

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

Chapter 39 Ecology and Evolution of Fungal-Bacterial Interactions

This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/1634115> since 2017-05-15T14:22:26Z

Publisher:

CRC Press Taylor & Francis Group

Published version:

DOI:10.1201/9781315119496-40

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

This is the author's final version of the contribution published as:

Olsson, Stefan; Bonfante, Paola; Pawlowska, Teresa E.. Chapter 39 Ecology and Evolution of Fungal-Bacterial Interactions. CRC Press Taylor & Francis Group. 2017. pp: 563-584.

in

The Fungal Community Its Organization and Role in the Ecosystem, Fourth Edition

The publisher's version is available at:

<http://www.crcnetbase.com/doi/pdf/10.1201/9781315119496-40>

When citing, please refer to the published version.

Link to this full text:

<http://hdl.handle.net/>

1 Ecology and Evolution of Fungal-Bacterial Interactions

2 Stefan Olsson¹, Paola Bonfante² and Teresa E. Pawlowska³

3 ¹Department of Plant & Environmental Sciences, University of Copenhagen, Copenhagen,
4 Denmark, sto@plen.ku.dk; ²Department of Life Sciences & Systems Biology, University of
5 Torino, Torino, Italy, paola.bonfante@unito.it; ³School of Integrative Plant Science, Plant
6 Pathology & Plant-Microbe Biology, Cornell University, Ithaca, NY 14853-5904, USA,
7 tep8@cornell.edu.

8

9

10 I. General Introduction

11 II. Definitions and Concepts

12 III. Non-Heritable Symbiotic Interactions

13 A. Introduction

14 B. *Candida albicans*-*Pseudomonas aeruginosa* antagonism

15 C. Mycophagy and biological control of fungi by bacteria

16 D. Fungal predation and dependence on bacteria

17 E. Highways carrying hyphae-associated bacteria

18 F. Mycorrhiza helper bacteria

19 G. Recognition and assembly of the non-heritable symbionts to form the fungal-bacterial 20 metaorganism

21 IV. Vertical Transmission and the Evolution of Mutualisms

22 V. Heritable Symbiotic Interactions

23 A. Introduction

24	B. Heritable facultative mutualisms
25	C. Heritable antagonisms
26	VI. Future Developments
27	A. Introduction
28	B. Novel tools to study fungal-bacterial metaorganisms
29	C. Physiological processes known from other host-symbiont systems
30	VII. Closing Remarks

31

32 **I. General Introduction**

33 The propensity of fungi to synthesize compounds active against bacteria (Broadbent 1966) and
34 the predilection of bacteria to produce antifungals (Kerr 1999) gave rise to a paradigm that
35 interactions between representatives of these two groups of organisms are of an antagonistic
36 nature. While, indeed, evidence for fungal-bacterial antagonisms is abundant (Espuny Tomas et
37 al. 1982; Leveau and Preston 2008; Susi et al. 2011; Palaniyandi et al. 2013; Pawlowska et al.
38 2012; Pliego et al. 2011), the recent accumulation of newly discovered associations in which
39 fungi cooperate with bacteria (Kobayashi and Crouch 2009; Frey-Klett et al. 2011) indicates that
40 such reciprocally beneficial interactions are more common than previously thought. As
41 functional and mechanistic aspects of many of these interdomain relationships were reviewed in
42 detail elsewhere (Grube and Berg 2009; Kobayashi and Crouch 2009; Peleg, Hogan, and
43 Mylonakis 2010; Frey-Klett et al. 2011; Martin and Schwab 2012; Scherlach, Graupner, and
44 Hertweck 2013), our discussion will focus on factors that contribute to their stability over
45 ecological and evolutionary time. We hope that, by directing attention to this important but
46 currently neglected aspect of fungal-bacterial interactions, we will inspire new directions of
47 research on the biology of these organisms.

48

49 **II. Definitions and Concepts**

50 We use the term **sympiosis** in the de Bary's sense of "the living together of unlike organisms",
51 without implications whether this relationship has positive or negative fitness consequences for
52 any of the interacting partners (Martin and Schwab 2012). Thus in terms of fitness outcomes, the
53 symbiosis can assume the forms of a **mutualism** (+/+), **commensalism** (+/0), and **antagonism**,

54 including **competition** (–/–), **amensalism** (–/0), **parasitism** and **predation/grazing** (–/+) (Lewis
55 1985). We doubt that strictly **neutral relationships** (0/0) exist among the symbiotic partners.
56 We recognize that practically all biota on the planet are components of stabile assemblages of
57 organisms, referred to as **metaorganisms** (Bosch and McFall-Ngai 2011). Although not ideal,
58 this term is reasonably well defined and increasingly coming into use (Trinchieri 2014; Biagi et
59 al. 2012). We employ it in our discussions of entities formed in the process and as a
60 consequence of fungal-bacterial interactions (Fig. 1). Thus it is the metaorganism that survives
61 in nature and changes over time due to evolution of its individual constituents, their composition,
62 and the roles in the metaorganism. It is important to note that fungal-bacterial metaorganisms
63 may be, in turn, components of higher-level metaorganisms comprising also plant or animal
64 hosts. We refer to the fungal constituents of the fungal-bacterial metaorganism as the **hosts** and
65 the bacterial partners as the **symbionts**. Both hosts and symbionts can be represented by a single
66 species, or they can each comprise a multi-species consortium in which different species interact
67 with each other. In terms of physical interface between the partners, bacterial symbionts can act
68 as **endobionts/endosymbionts** living intracellularly inside the hyphae, or as
69 **ectobionts/epibionts/ectosymbionts/episymbionts** associated with the surface of the hyphae or
70 in the close vicinity of the hyphae, often in biofilms consisting of several layers of bacteria held
71 together by a matrix. Metaorganism formation can take several routes. Most known associations
72 of fungi with bacteria are **non-heritable**, with bacterial symbionts assembled by each generation
73 of the host *de-novo* from the environment. In contrast, **heritable** bacterial symbionts are
74 transmitted vertically from the host parent to the next generation of the fungal-bacterial
75 metaorganism. Vertical transmission can be either strict/exclusive, or mixed, *i.e.* punctuated by
76 instances of horizontal transmission in which bacteria spread between host individuals of the

77 same generation. Bacterial symbionts can be free-living. They can also be confined to their
78 eukaryotic host's intracellular environment and have no extracellular state (**obligate**
79 **endobacteria**), or capable of living both in fungal cells and in extracellular environments
80 (**facultative endobacteria**). Finally, mutualistic symbionts can be divided based on their effects
81 on host survival into **essential** and **nonessential**.

82 Because of varying levels of integration and complexity, understanding of fungal-
83 bacterial metaorganisms is at present in its infancy. We believe that many facets of this
84 biological complexity can be studied and framed conceptually using the existing ecology and
85 evolution tools and theory. For example, some spontaneously formed fungal-bacterial
86 associations can be explained by **ecological fitting**, in which organisms establish novel relations
87 with other species thanks to the traits that they already possess when they encounter their new
88 partners (Janzen 1985). Such relationships often develop in man-made or disturbed
89 environments. Other interactions are expected to be products of prolonged reciprocal selection
90 that tie individual partner taxa or guilds of interacting partners into ecologically and
91 evolutionarily stable alliances. One of the approaches for organizing the knowledge on how
92 these entities are structured internally and coexist in ecosystems involves reconstruction of
93 **symbiotic networks** to inventory and display interactions among taxa within and across different
94 metaorganisms. In addition to being an inventory of taxa and their interactions, the networks are
95 expected to offer insights into the coevolutionary processes that shape the diversity of both
96 metaorganism constituents and metaorganisms themselves (Bascompte and Jordano 2013). In
97 particular, they represent patterns of selection operating among genetically variable multi-species
98 groups in which the species convergently adapt and specialize on a suite of symbiotic traits
99 rather than directly on other species (Thompson 2005). While, historically, symbiotic networks

100 have been used to represent interactions in mutualisms (Bascompte and Jordano 2013), they can
101 also accommodate interactions with negative fitness outcomes. Another framework that can help
102 explore and conceptualize fungal-bacterial interactions is the **geographic mosaic of coevolution,**
103 **GMC,** model (Thompson 2005). According to this model, partners interact across their
104 geographic ranges. In some locations, known as **coevolutionary hot spots**, they are subjected to
105 reciprocal selection. In others, known as **coevolutionary cold spots**, local selection is not
106 reciprocal. Several factors, including gene flow, genetic drift, mutations, migration, and local
107 extinctions, contribute to variation in the patterns of natural selection between the habitats.
108 These predictions can be readily translated into set of questions to guide investigations of fungal-
109 bacterial interactions (Gomulkiewicz et al. 2007).

110 While many fungal-bacterial interactions remain ambiguous in terms of fitness outcomes,
111 the vast majority of them are either undisputed antagonisms or mutualisms. The astounding
112 ubiquity and prevalence of antagonistic interactions present in all ecosystems is related to the
113 fact that living organisms represent excellent sources of energy and nutrients, which otherwise
114 are available in limiting quantities (Thompson 2014). In fact, even mutualisms are viewed as
115 reciprocal exploitations that nonetheless provide net benefits to each partner (Herre et al. 1999).
116 Moreover, despite their fundamental significance to the evolution and functioning of the
117 biosphere, the mechanisms that promote the initial establishment and evolutionary stability of
118 mutualisms are not fully explored. Like antagonisms, mutualisms can form instantaneously as a
119 consequence of ecological fitting (Janzen 1985; Hom and Murray 2014). They can be also
120 products of extensive reciprocal selection between the partners that initially interacted as either
121 antagonists or commensals (Aanen and Bisseling 2014). Conflicting interests of the interacting
122 partners, manifested by accepting benefits without reciprocating, make mutualisms vulnerable to

failures. Yet, their evolutionary persistence suggests that certain mechanisms could ensure mutualism stability (Trivers 1971). Several theoretical models have been proposed to explain evolutionary stability of mutualisms. They include: (1) **byproduct cooperation** (Connor 1986; Sachs et al. 2004), (2) the **iterated prisoner's dilemma, IPD**, model with the “**tit-for-tat**” strategy (Axelrod and Hamilton 1981; Doebeli and Knowlton 1998; Sachs et al. 2004), (3) **partner-fidelity feedback, PFF** (Bull and Rice 1991; Sachs et al. 2004; Weyl et al. 2010), (4) **partner choice** (Bull and Rice 1991; Noë and Hammerstein 1994; Sachs et al. 2004), and (5) **compensatory evolution/addiction** (Aanen and Hoekstra 2007). (1) Byproduct cooperation involves interactions in which a focal partner receives a byproduct benefit from a donor and natural selection shapes the focal partner to maximize these benefits by being cooperative toward the donor (Connor 1986; Sachs et al. 2004). (2) The IPD model with the “tit-for-tat” strategy applies to systems in which two partners, who engage in a series of interactions, are able to vary their behavior in each interaction according to a partner's previous action (Axelrod and Hamilton 1981; Doebeli and Knowlton 1998; Sachs et al. 2004; Weyl et al. 2010). Cooperation is maintained only when partners reciprocate in kind. Non-cooperative individuals are sanctioned by their partners through termination of cooperation. (3) Like IPD, the PFF model applies to systems in which two partners interact repeatedly. However, in PFF, fitness gains derived from cooperation by one partner feed back to the other partner, thus the partner who fails to cooperate harms its own fitness (Bull and Rice 1991; Sachs et al. 2004; Weyl et al. 2010). (4) Unlike IPD and PFF, the partner choice model involves interactions of a focal individual with multiple trading partners who are reciprocated based on the quality of goods and services offered, with the most cooperative partner receiving the highest compensation (Sachs et al. 2004; Kiers et al. 2011). (5) The mechanism of compensatory evolution/addiction is expected to operate in

146 mutualisms that evolved from antagonistic interactions, in host populations exposed initially to a
147 parasitic symbiont (Aanen and Hoekstra 2007). Under parasite pressure, host mutants are
148 favored that compensate for harmful effects of the parasite and thus suffer less damage. Once
149 such compensatory mutations are fixed, they may become deleterious to the host in the absence
150 of the parasite. As a consequence, a host population with such compensatory mutations will
151 become dependent on the presence of the parasite, leading ultimately to a conversion of an
152 antagonistic interaction into a stable mutualism.

153 For the sake of clarity, we divided our discussion of fungal-bacterial symbioses into
154 sections devoted to systems in which partners are assembled *de novo* in each generation versus
155 associations in which partners are transmitted together from generation to generation and
156 interactions are heritable. We also discussed the role of vertical transmission in evolution of
157 mutualisms from antagonisms. Finally, we suggested tools and future directions for studying
158 fungal-bacterial symbioses.

159

160 **III. Non-Heritable Symbiotic Interactions**

161 **A. Introduction**

162 All basic types of relationships, *i.e.* mutualisms, commensalisms, and antagonisms, can be found
163 among non-heritable fungal-bacterial symbioses. For some of them detailed knowledge is
164 available, others will be mentioned only briefly. Some bacteria associate directly with fungal
165 hyphae (Baschien et al. 2009; Cuong et al. 2011) and form biofilms on their surfaces (Simon et
166 al. 2015; Pion et al. 2013; Scheublin et al. 2010). These epibionts live in the hyphosphere, the
167 volume around hypha influenced by the hyphal presence (**Errore. Riferimento a**
168 **collegamento ipertestuale non valido**). The bacterial symbionts can be antagonistic, as is

Eliminato: Staněk 1984

169 typical for bacteria used for biocontrol of fungal pathogens (Mela et al. 2011; Cuong et al. 2011;
170 Jochum, Osborne, and Yuen 2006; Mathioni et al. 2013). They can also act as mutualists (Nazir,
171 Tazetdinova, and van Elsas 2014). However, it seems that there is a limited number of
172 fungiphilic bacterial taxa, *i.e.* taxa adapted to the mycosphere, that are involved in fungal-
173 bacterial symbioses (Lyons et al. 2005; Warmink, Nazir, and van Elsas 2009; Simon et al. 2015;
174 Baschien et al. 2009; Scheublin et al. 2010). Finally, some non-heritable interactions are quite
175 unexpected and thought provoking, like those formed by bacteria-farming fungi (Pion et al.
176 2013), or bacterivorous nematodes and nematophagous fungi (Wang et al. 2014; Hsueh et al.
177 2013).

178

179 **B. *Candida albicans*-*Pseudomonas aeruginosa* antagonism**

180 Because of their significance to human health, interactions between *Candida albicans* and
181 *Pseudomonas aeruginosa* attracted a lot of attention, which, in turn, yielded important insights
182 into the molecular mechanisms that underlie the coexistence of these two organisms in the
183 context of human disease (Peleg, Hogan, and Mylonakis 2010). *C. albicans* is a commensal
184 yeast found in the normal microbial flora of human oral, digestive, or vaginal mucosa (McManus
185 and Coleman 2014). It is acquired at birth or during physical contact. Factors affecting the
186 mucosal microbiome, such as the use of antibiotics, hormonal imbalance, or diet, can induce
187 non-life threatening *C. albicans* infections of mucosal surfaces, candidiasis (Scully, el-Kabir, and
188 Samaranayake 1994). In severely ill and immunocompromised individuals, *C. albicans* can
189 spread into the blood stream causing invasive and often fatal candidaemia (Eggimann, Garbino,
190 and Pittet 2003). *C. albicans* invasions of host tissues are associated with a morphogenic switch
191 from yeast-like to filamentous growth, which can be induced by changes in environmental

192 conditions, such as shifts in temperature and pH (Berman and Sudbery 2002).

193 *C. albicans* history is intimately linked with the history of humans. Phylogenetic data
194 suggest that its diversification occurred ~3 to 16 MYA and coincided with the evolution of early
195 hominids (Lott et al. 2005). Moreover, it is believed that humans are the main environmental
196 reservoir of *C. albicans* (Angebault et al. 2013). In contrast to *C. albicans*, *P. aeruginosa* is a
197 ubiquitous microbe that can be isolated from diverse environments, including humans (Lister,
198 Wolter, and Hanson 2009). However, unlike *C. albicans*, it is rarely a member of the normal
199 microbial flora in humans. Instead, it is a causal agent of community-acquired and, more often,
200 nosocomial infections in individuals who are immunocompromised or suffered a breach in
201 cutaneous or mucosal barriers. The recently observed rise in opportunistic *P. aeruginosa*
202 infections appears to be related to the ability of this microbe to rapidly develop multidrug-
203 resistant phenotypes.

204 Mixed infections in which *P. aeruginosa* coexists with *C. albicans* often occur in patients
205 with burn wounds (Gupta et al. 2005) and chronic lung diseases (Hughes and Kim 1973). In
206 such infections the two organisms display an array of antagonistic interactions centered on
207 competition for the host resources and mediated by several mechanisms. For example, *C.*
208 *albicans* responds to the *P. aeruginosa* quorum-sensing signal 3-oxo-C12 homoserine lactone
209 (3OC12HSL) as well as its 12 carbon chain analogs C12HSL and dodecanol with the inhibition
210 of yeast cell filamentation and conversion of previously formed filaments to yeast cells (Hogan,
211 Vik, and Kolter 2004). These are likely defensive responses, as *P. aeruginosa* can attach to the
212 surface of *C. albicans* hyphae and kill them through the action of phospholipase C and
213 phenazines; yeast cells are not susceptible to *P. aeruginosa* attachment (Hogan and Kolter 2002;
214 Gibson, Sood, and Hogan 2009).

215 Initially, the morphogenic effects of *P. aeruginosa*-derived C12 compounds on *C. albicans*
216 were considered to be purely coincidental as these molecules share structural similarity with
217 farnesol. Farnesol is the C12 autoregulatory molecule that controls yeast-to-hypha transition in
218 *C. albicans* (Hogan, Vik, and Kolter 2004) by modulating cyclic AMP signaling through direct
219 inhibition of the adenylate cyclase activity (Davis-Hanna et al. 2008; Lindsay et al. 2012; Hall et
220 al. 2011) and suppressing filamentation of yeast cells (Hornby et al. 2001). Recent studies
221 revealed that despite structural similarities among the C12 HSLs and their analogs, only
222 3OC12HSL mimics farnesol's activity by interacting with the adenylate cyclase. Another C12
223 compound, dodecanol prevents yeast-to-hypha transition through a different mechanism
224 involving the transcriptional hyphal suppressor Sfl1p (Hall et al. 2011). Interestingly, dodecanol
225 shares structural similarity with a diffusible signal factor of *Burkholderia cenocepacia* (Hall et
226 al. 2011), which also interferes with *C. albicans* filamentation (Boon et al. 2008). Like *P.*
227 *aeruginosa*, representatives of the *B. cepacia* complex frequently coexist and interact
228 antagonistically with *C. albicans* in mixed infections of patients who are immunocompromised
229 and suffer chronic lung disease (Kerr 1994). Notably, however, *C. albicans* does not seem to
230 respond to C8 HSL, the major quorum-sensing signal produced by *B. cepacia* (Hogan, Vik, and
231 Kolter 2004; Boon et al. 2008).

232 In addition to autoregulation of fungal morphogenesis, farnesol plays a role in
233 interactions with bacterial antagonists by inhibiting biosynthesis of the *P. aeruginosa* quinolone
234 signal (PQS) and the PQS-controlled biosynthesis of the pyocyanin siderophore virulence factor
235 (Cugini et al. 2007). Moreover, *C. albicans* interferes with *P. aeruginosa* signaling and
236 metabolite production. It can also inhibit virulence of *P. aeruginosa* in mice by inhibition of
237 bacterial pyochelin and pyoverdine siderophore biosynthesis (Lopez-Medina et al. 2015).

While the structural similarity of compounds that suppress yeast-to-hypha transition in *C. albicans* may suggest ecological fitting, the diversity of the morphogenic mechanisms utilized by *C. albicans* to respond to these bacterial C12 signal molecules as well as the complex interplay of inhibitory interactions between *C. albicans* and its bacterial antagonists suggest that these organisms may have been undergoing reciprocal selection within the context of human disease. This process is expected to intensify with the continued increase in the number of patients who require immune system suppression.

C. Mycophagy and biological control of fungi by bacteria

Fungal hyphae are a potential nutrient and energy source for bacteria. Some bacteria seem to be specialized in feeding on fungi and have been considered mycophagous (Leveau and Preston 2008). They have been studied mainly as potential biological control agents aimed toward plant pathogenic fungi (Jochum, Osborne, and Yuen 2006; Yoshida et al. 2012; Selin et al. 2010). These antagonistic bacteria can kill the fungus using a combination of enzymes and antifungal compounds. A well-studied and interesting antifungal compound produced by *Lysobacter* is HSAF (heat-stabile antifungal factor), a hybrid PKS-NRPS inhibiting the fungal acyl-CoA-dependent ceramide synthase, an enzyme unique to filamentous fungi (Li et al. 2008; Yu et al. 2007). This inhibition affects the formation of lipid rafts that are important for proper fungal exocytosis and endocytosis (Li et al. 2006; Alvarez, Douglas, and Konopka 2007).

Importantly, most potential biological control organisms have been selected for their ability to produce antifungal compounds on agar plates but it is unclear if they also use the fungus as an energy or carbon source or, indeed, if the same inhibiting compounds are active as biocontrol agents in the natural environments (Thrane et al. 2000). Moreover, it is not necessary

261 for mycophagous bacteria to lyse the fungal hyphae in order to parasitize the fungus, proliferate,
262 and inhibit the fungus efficiently. Some bacteria kill the fungus and multiply without penetrating
263 its cell walls, while others proliferate without any negative effects to the fungus (Cuong et al.
264 2011).

265 With the advent of transcriptomics and proteomics, new insights have been gained into
266 these antagonistic of interactions. For example, dual transcriptomic studies of both the fungus
267 and the bacterium challenging each other on agar plates focused on interactions between
268 *Aspergillus niger* and *Collimonas fungivorans* (Mela et al. 2011) as well as *Rhizoctonia solani*
269 and *Serratia plymuthica* (Gkarmiri et al. 2015; Neupane et al. 2015). In these studies, the
270 partners were not allowed to come into physical contact but could exchange metabolites, and in
271 both cases the portion of the fungal colony that was transcriptionally profiled was the one
272 adjacent to the inhibition zone. Both studies found that the fungi reacted by upregulating defense
273 responses (detoxification, efflux pumps), changes to membrane permeability, and increased
274 oxalate production. In contrast, the only response common in bacteria was the upregulation of
275 genes involved in production of secondary metabolites (Mela et al. 2011; Gkarmiri et al. 2015).
276 The two interactions were in many other ways quite different. The *Aspergillus-Collimonas*
277 interaction was mainly characterized by a competition for nitrogen (Mela et al. 2011), while the
278 *Rhizoctonia-Serratia* interaction involved a mutual chemical warfare, as both the fungus and the
279 bacteria upregulated transcription of genes responsible for secondary metabolites/toxins and
280 defenses (Gkarmiri et al. 2015; Neupane et al. 2015).

281 Another example of fungal-bacterial antagonistic interactions comes from *Magnaporthe*
282 *oryzae* transcriptional responses after direct contact with *Lysobacter enzymogenes*, both a wild
283 type (WT) strain and a mutant strain deficient in virulence (Mathioni et al. 2013). Four

284 *Magnaporthe* genes were induced at 3 hours by both WT and mutant bacteria, and two of these
285 were known stress response genes (a laccase and a beta-lactamase). The hypothesis that WT *L.*
286 *enzymogenes* is capable of turning off fungal defenses while the mutant could not was used to
287 interpret the data. A total of 463 *Magnaporthe* genes were down-regulated by WT *L.*
288 *enzymogenes*. Of these genes, 100 were up-regulated in interaction with the non-virulent mutant
289 and assumed to be genes involved in the fungal general response/defense against bacteria. These
290 genes are predicted to have roles in carbohydrate metabolism, cellular transport and stress
291 response (Mathioni et al. 2013).

292 The examples discussed above offer glimpses into the vast and complex world of
293 metabolic activities involved in trophic interactions between bacteria and fungi, as we are only
294 starting to uncover and understand these food webs. Clearly, further sustained efforts are needed
295 to identify the players and understand the flows of energy and nutrients that support the
296 communities of fungi and bacteria forming such trophic networks.

297

298 **D. Fungal predation and dependence on bacteria**

299 Fungi can attack, degrade, and use bacteria as nutrient sources (Barron 1988; Barron 2003).
300 These capabilities have mainly been noted in basidiomycete wood decomposers, with nitrogen
301 limitation being the main trigger of fungal predation on bacteria (Barron 2003). Wood
302 decomposing fungi have profound effects on bacterial composition of the substrate they colonize
303 and the bacterial composition becomes characteristic for the fungal species colonizing the
304 substrate (Tornberg, Bååth, and Olsson 2003; de Boer et al. 2005). Along similar lines, nitrogen
305 fixation by bacteria seems to be important in wood decay and it has been suggested that nitrogen-
306 fixing bacteria grow on the low molecular carbon released by the wood decaying fungi and that

307 the fungus then selectively harvests and degrades some of the bacteria as a source of nitrogen (de
308 Boer and van der Wal 2008). This idea has found support in a study of the *nifH* dinitrogenase
309 reductase diversity in dead wood, where a non-random co-occurrence pattern between nitrogen-
310 fixing bacteria and fungal species was detected, indicating specific interactions between fungi
311 and bacteria (Hoppe et al. 2014). Similarly, *Rhizobium*-type nitrogen-fixing bacteria can form
312 biofilms on fungi and this seems to affect the activity and survival of both organisms
313 (Seneviratne and Jayasinghearachchi 2003; Seneviratne et al. 2008).

314 Of relevance to the observations on the trophic interactions between fungi and bacteria is
315 the concept of bacteria farming by fungi, which was recently introduced to describe the
316 relationship between the fungus *Morchella crassipes* and *Pseudomonas putida* (Pion et al. 2013).
317 *M. crassipes* disperses bacteria, rears them on fungal exudates as well as harvests and
318 translocates bacterial carbon (Pion et al. 2013). It is possible that a similar mechanism of
319 bacteria farming by fungi can be behind the observed interactions between nitrogen-fixing
320 bacteria and fungi and could account for the apparent stability of the interactions.

321 Finally, not all trophic interactions involving fungi and bacteria are antagonistic. An
322 example of a more complex interaction comes from the cow dung-inhabiting bacterium
323 *Stenotrophomonas maltophilia*. These bacteria are consumed by the bacterivorous nematode
324 *Caenorhabditis elegans*. As a defense mechanism, the bacteria secrete urea that mobilizes the
325 nematophagous fungus *Arthrobotrys oligospora* to respond to the nematode presence and
326 eliminate them. Nematode elimination is accomplished by the increased production of sticky
327 hyphal nets that trap and kill nematodes, which are then consumed by the fungus (Wang et al.
328 2014; Hsueh et al. 2013).

329 Like with trophic interactions in which bacteria feed on fungi, fungal predation and
330 farming of bacteria are most likely widespread and underappreciated features of terrestrial
331 ecosystems. While some of them can be readily reproduced under laboratory conditions, others
332 need to be studied *in situ* in their natural environments to understand how they connect to more
333 conventional food webs.

334

335 **E. Highways carrying hyphae-associated bacteria**

336 Fungal hyphae expanding in and through unsaturated soil can spread in a soil volume easier than
337 bacteria, as they can bridge over aerial pores and other hydrophobic regions (Kohlmeier et al.
338 2005). The surfaces of the fungus assimilatory hyphae are hydrophilic and thus the fungal
339 hyphae form hydrophilic tracks through soil. These tracks are referred to as **fungal highways**
340 that the bacteria can follow and are generally regarded as beneficial to both the host and the
341 bacterial symbionts (Kohlmeier et al. 2005). The fungal highways have been studied in relation
342 to dissemination of pollutant-degrading bacteria (Kohlmeier et al. 2005; Furuno et al. 2010). In
343 particular, it has been shown that the fungal hyphae might not just help to spread the bacteria but
344 could also function as conduits of pollutants to bacteria (Banitz et al. 2014; Furuno et al. 2010;
345 Wick et al. 2007). In this respect, substrate is channeled from a source along the hyphae to
346 bacteria that are associated with these hyphae. The fungal host seems to nourish the bacterial
347 symbionts inhabiting and spreading on the highways (Bravo et al. 2013; Nazir et al. 2013). The
348 number of bacterial taxa associating and travelling along the fungal highways is probably a
349 combination of selection for the specific prevalent conditions, available substrates, and also by
350 direct activities of the host, *e.g.* a consequence of mutualist recognition or absence of parasite
351 recognition. Bacterial motility by flagella as well as other types of motility have been suggested

352 as a common characteristic of bacteria travelling on the fungal highways (Bravo et al. 2013).
353 Among bacterial taxa especially common in the hyphosphere is the genus *Burkholderia* (Suárez-
354 Moreno et al. 2012). Interestingly, the same genus is also prominent among fungal
355 endosymbionts (see sections below). Fungus-derived oxalate and glycerol have been shown to
356 feed both mutualistic and parasitic bacterial symbionts living and spreading on the fungal
357 highways (Bravo et al. 2013; Nazir et al. 2013). It has also been shown that some bacteria that
358 migrate as “hitchhikers” along fungal highways can only do this if other bacteria have paved the
359 way for them (Warmink et al. 2011). Interestingly, such facilitation does not apply to all bacteria
360 (Warmink et al. 2011). Thus there appear to be three categories of bacteria in relation to
361 movement along fungal hyphae: (1) independent travelers that manage to set up the conditions
362 with the fungal hosts necessary for travel, (2) hitchhikers dependent on the simultaneous
363 presence of the independent travelers, and (3) non-travelers, either not having the properties,
364 such as motility, to move along the fungal highway, or being inhibited by the fungal host and/or
365 the first two types of travelers.

366 The potential importance of fungal highways to the soil bacteria suggests that these
367 interactions may be a common and, until recently, overlooked feature of soil ecosystems.
368 Consequently, the diversity of both fungi that serve as the thoroughfares and their bacterial
369 travelers requires in-depth surveying. The approach of symbiotic network reconstruction appears
370 to be a natural starting point for understanding the rules that govern highway usage. Importantly,
371 while bacterial travelers clearly benefit from highway availability, as it improves their mobility
372 in the soil and may offer a source of nourishment, it is unclear whether fungi receive any benefits
373 from this interaction. Is it a mutualism or an interaction in which the fungal partner remains
374 unaffected or perhaps even harmed?

375

376 **F. Mycorrhiza helper bacteria**

377 Mycorrhizal fungi form with the roots of terrestrial plants symbiotic associations of distinct

378 morphologies and functions, collectively referred to as mycorrhizas (Smith and Read 2008). In

379 the most common among them, ecto- and arbuscular mycorrhizas, fungi facilitate plant mineral

380 nutrient uptake from the soil in return for photosynthetic carbon. As a consequence, these

381 symbioses are of great significance in both natural and managed ecosystems, with a particular

382 impact on agriculture and forestry. Current observations indicate that mycorrhizas are, in fact,

383 complex multipartner interactions (Bonfante and Anca 2009), due to the presence of bacteria that

384 can be either loosely or tightly associated with mycorrhizal fungi (Jansa, Bukovská, and

385 Gryndler 2013; Bianciotto et al. 2001; Perotto and Bonfante 1997). Garbaye (1994) pioneered

386 the work on these associations with the now widely accepted term **mycorrhiza helper bacteria**,

387 **MHB**, which defines bacteria that help mycorrhizal establishment. Since the time of MHB

388 discovery and thanks to the advent of the omics era, new knowledge and insights have

389 accumulated, with a particular focus on the microbiota present in the rhizosphere and endosphere

390 of poplar (*Populus*).

391 As a host for both ecto- and arbuscular mycorrhizal fungi (AMF), poplar is an excellent

392 model for understanding interactions that govern establishment and functioning of mycorrhizal

393 symbioses, including the role of MHB. For example, the genomes of 21 strains of *Pseudomonas*

394 isolated from the *Populus deltoides* rhizosphere and endosphere have been sequenced (Brown et

395 al. 2012), giving rise to extensive genetic and bioinformatic resources. As a further step, these

396 bacterial isolates were screened for MHB effectiveness expressed as the effects on the *Laccaria*

397 *bicolor* S238N growth rate, mycelial architecture, transcriptional changes and symbiosis with

398 three *Populus* lines, *P. tremula* × *alba*, *P. trichocarpa*, and *P. deltoides*. Nineteen of the studied
399 isolates had positive impact on *L. bicolor* growth (Labbé et al. 2014). Interestingly, one strain
400 promoted high root colonization also in *P. deltoides*, which is otherwise poorly colonized by *L.*
401 *bicolor*. In this context, the genome of a MHB isolate of *Pseudomonas fluorescens* BBc6R8 will
402 be of great advantage in identifying the helper traits (Deveau et al. 2014).

403 Prokaryotes are associated not only with the extraradical hyphae of mycorrhizal fungi,
404 but also with ectomycorrhizal roots and sporocarps, *i.e.*, the fruiting bodies formed by
405 ectomycorrhizal ascomycetes and basidiomycetes, suggesting that they may accompany
406 mycorrhizal fungi during the various steps of their life cycle. Because of their economic
407 significance, *Tuber* sporocarps have become a model to understand a role that truffle-associated
408 bacteria play in several still poorly understood aspects of truffle development, from fruiting body
409 formation to aroma production. Similarly, the appearance of the “brûlé”, an area devoid of
410 vegetation around the *Tuber* host plants and where the fruiting bodies of *T. melanosporum* are
411 usually collected, is a feature with a clear ecological impact but largely unknown causes. For
412 example, the examination of direct fungal-fungal interactions (Napoli et al. 2010), together with
413 DGGE and DNA microarray analyses of 16S rRNA gene fragments (Mello et al. 2013), revealed
414 that the bacteria and archaeal communities strongly differ between the inside versus outside of
415 the brûlé area. The groups that were most severely affected by the black truffle included
416 Firmicutes, several genera of Actinobacteria, and a few Cyanobacteria. One of the mechanisms
417 responsible for this pattern could be the capacity of truffles to release volatile organic
418 compounds (Splivallo et al. 2011). Intriguingly, Splivallo et al. (2015) found that sulphur-
419 containing volatiles, such as thiophene derivatives characteristic of *T. borchii* fruiting bodies, are
420 products of the bacteria-mediated biotransformation of non-volatile precursor(s) into volatile

421 compounds. Moreover, the α - and β -proteobacteria-dominated community of *T. borchii* was able
422 to produce thiophene volatiles from *T. borchii* fruiting body extract, irrespective of their isolation
423 source (truffle or other sources).

424 The complexity of interactions between fungi and both MHB and sporocarp-associated
425 bacteria makes them uniquely difficult to study. However, the tools of symbiotic network
426 construction and testing the applicability of the GMC model to these systems may provide
427 structured approaches to make rapid progress in understanding of these systems.

428

429 **G. Recognition and assembly of the non-heritable symbionts to form the fungal-bacterial**
430 **metaorganism**

431 Both plant and animal epithelial surfaces coming in contact with bacteria share a similar problem
432 in that they should actively select for beneficial/commensal bacteria and discourage the
433 colonization by antagonists (McFrederick et al. 2012; Artis 2008; Ausubel 2005; Zamioudis and
434 Pieterse 2012). Innate immunity recognition of bacterial cues as MAMPs (microbial associated
435 molecular patterns) plays a key role in this selection in both plants and animals (Artis 2008;
436 Nürnberger et al. 2004). However, the immune reaction is balanced so as not to kill eventual
437 beneficial bacteria, as is done in tissues not normally colonized by bacteria (Artis 2008;
438 Zamioudis and Pieterse 2012). Fungal hyphae growing in most natural environments face a
439 similar need to promote the beneficial and inhibit the antagonistic microbes. Fungal reactions to
440 a bacterial MAMP have been demonstrated (Xu et al. 2008), the existence of innate immunity
441 type recognition has been suggested (Paoletti and Saupe 2009; Paoletti, Saupe, and Clavé 2007),
442 and recently transcriptomic innate immunity type responses have been found in fungi (Ipcho et
443 al. 2016). Fungal innate immunity is thus most likely involved in the active selection for

444 beneficial bacteria as it is in other eukaryotic hosts. The main mechanisms of such selection
 445 involve production of antibiotics/secondary metabolites, selective provisioning of nutrients to the
 446 beneficial bacteria (Huang et al. 2014; Hartmann et al. 2009; Ramírez-Puebla et al. 2013; Oozeer
 447 et al. 2013; Scholtens et al. 2012), and creating conditions unfavorable for pathogens (Kai-
 448 Larsen, Gudmundsson, and Agerberth 2014; Markel et al. 2007; Ramírez-Puebla et al. 2013).
 449 The selective recruitment of beneficial bacteria is further helped by their either passive or active
 450 transfer between host generations (Oozeer et al. 2013; Scholtens et al. 2012; Ramírez-Puebla et
 451 al. 2013), thus resembling heritable transmission.

452 Interestingly, several mechanisms appear to be shared by diverse host symbiont-systems
 453 (Table 1). For example, the gut epithelium, the rhizoplane, and the hyphosphere are typically
 454 low-pH environments and this pH decrease is stimulated further by bacterial presence (Ramírez-
 455 Puebla et al. 2013), a condition also shared by animal tissue inflammation (Rajamäki et al.
 456 2013). Another key reaction in innate immunity is active sequestration of iron by the plant and
 457 animal hosts (Markel et al. 2007; Ganz 2009; Ong et al. 2006; Lemanceau et al. 2009). As a
 458 consequence, iron levels are much depleted both in the rhizosphere (Lemanceau et al. 2009) and
 459 in the gut (Ganz 2009; Markel et al. 2007; Ong et al. 2006). Beneficial bacteria appear to be
 460 adapted to such low-iron conditions and display either very low demand for iron, as the probiotic
 461 *Lactobacillus plantarum* (Archibald 1983), or have very efficient siderophores, like many plant
 462 growth-promoting rhizobacteria (Beneduzi, Ambrosini, and Passaglia 2012). Interestingly, most
 463 genes involved in iron acquisition are also rapidly upregulated in *Fusarium graminearum* in
 464 response to bacterial MAMPs (Ipcho et al. 2016). Finally, beneficial bacteria in the rhizosphere
 465 are stimulated by plant rhizosphere-specific sugars, like raffinose and sucrose (Huang et al.
 466 2014), which are generally not present in the soil, while beneficial gut bacteria in mammals are

stimulated by fructans (Oozeer et al. 2013; Scholtens et al. 2012). Fungi interacting with bacteria have been also shown to secrete carbon sources, such as oxalate (Scheublin et al. 2010), glycerol (Nazir et al. 2013) and trehalose (Deveau et al. 2010), which could possibly serve similar selective functions for beneficial bacteria.

The mechanisms responsible for the assembly of fungal-bacterial metaorganisms thus appear to have parallels with other eukaryotic-bacterial metaorganisms and much can be learnt from these other systems. Because fungi are relatively easy to study and manipulate genetically, there is a great potential for rapid progress in understanding the fungal-bacterial interactions. Importantly, we expect that all horizontally transmitted bacterial symbionts as well as the bacteria engaged in heritable facultative mutualisms with fungi need to employ these mechanisms when initiating the interaction with their hosts.

IV. Vertical Transmission and the Evolution of Mutualisms

Because of its role in coupling of partner reproductive interests, vertical transmission is widely recognized as a mechanism that stabilizes mutualisms under several evolutionary models, including byproduct cooperation (Connor 1986; Sachs et al. 2004), IPD with the “tit-for-tat” strategy (Axelrod and Hamilton 1981; Doebeli and Knowlton 1998; Sachs et al. 2004), PFF (Bull and Rice 1991; Sachs et al. 2004; Weyl et al. 2010), and compensatory evolution/addiction (Aanen and Hoekstra 2007). In addition, vertical transmission is expected to play an important part in evolution of antagonistic interspecific interactions into mutualisms (Yamamura 1993). Evolutionary theory predicts that a symbiotic system will transition from antagonism to mutualism once a parasite is able to dominate the co-evolutionary race with the host and achieve a rate of vertical transmission that enables efficient reciprocal selection between the partners

490 (Yamamura 1993) (Fig. 2). If the increase in the rate of symbiont vertical transmission is
491 accompanied by the development of host abilities to complement its metabolism using symbiont
492 metabolites, a byproduct mutualism is expected to evolve (Yamamura 1993).

493 While the model that explains the evolution of mutualisms from antagonisms through
494 changes in the rates of symbiont transmission is rather straightforward (Yamamura 1993), the
495 actual mechanisms that permit symbiont vertical transmission remain elusive as nearly all known
496 heritable endosymbionts are uncultivable (Moran, McCutcheon, and Nakabachi 2008) and many
497 hosts are unable to survive without their endobacteria. In this context, the rice seedling blight
498 fungus *Rhizopus microsporus* and its endosymbiont *Burkholderia rhizoxinica* offer an
499 unprecedented opportunity to understand the evolution of mutualisms from antagonisms
500 (Partida-Martinez and Hertweck 2005; Lackner and Hertweck 2011). In this system, the
501 endobacteria reside directly within the fungal cytoplasm (Partida-Martinez et al. 2007). Their
502 elimination with antibiotics abolishes fungal ability to form asexual sporangia and
503 sporangiospores (Partida-Martinez et al. 2007), suggesting that endobacteria not only gained
504 control of their own transmission rate but also of the reproductive success of the fungus, a
505 pattern consistent with the compensatory evolution/addiction model of mutualism evolution
506 (Aanen and Hoekstra 2007). In addition to controlling the rate of own vertical transmission by
507 rendering fungal reproduction dependent on their presence, the endobacteria produce a macrolide
508 metabolite that is processed by the host to form a highly potent antimitotic toxin called rhizoxin
509 (Scherlach et al. 2012). The toxin is active in rice seedlings, where it causes the blight disease
510 (Lackner, Partida-Martinez, and Hertweck 2009). In addition, rhizoxin is believed to facilitate
511 competitive interactions of the *Rhizopus* host with fungi that are sensitive to it. Such positive
512 effects of the symbiont-derived metabolite on host fitness suggest that the *Rhizopus*-

513 *Burkholderia* symbiosis can be viewed as a byproduct mutualism, in addition to being an
514 example of the addiction model. The *Rhizopus* host, like other Mucorales, is protected from
515 harmful effects of the toxin by a specific mutation in its β -tubulin gene (Schmitt et al. 2008).
516 The presence of this protective mutation across other Mucorales suggests that it was a
517 preadaptation that allowed *Rhizopus* for entering a byproduct mutualism with the *Burkholderia*
518 endobacteria.

519 The 3.75 Mb genome of *B. rhizoxinica* appears to be moderately sized compared to free-
520 living *Burkholderia* with genomes of 8 – 9 Mb (Winsor et al. 2008), but is considerably larger
521 than the genomes of closely related endosymbiotic β -proteobacteria, including *Candidatus*
522 *Glomeribacter gigasporarum* (1.72 Mb) (Ghignone et al. 2012), the unnamed endosymbiont of
523 *Mortierella elongata* (2.65 Mb) (Fujimura et al. 2014), and *Ca. Tremblaya princeps* (0.14 Mb)
524 (McCutcheon and von Dohlen 2011). Such reductions in the endosymbiont genome size are
525 associated with the process of adaptation to the host cellular environment (McCutcheon and
526 Moran 2012). Nevertheless, the *Burkholderia* endobacteria of *Rhizopus* remain not only
527 metabolically independent of the host and but also capable of invading compatible hosts *de novo*
528 (Moebius et al. 2014). In particular, the release of bacterial chitinolytic enzymes and chitin-
529 binding proteins enables breaching of fungal cell walls and the initiation of the invasion process
530 (Moebius et al. 2014). In turn, the survival and proliferation of *B. rhizoxinica* inside fungal cells
531 appears to depend on the activity of the type III secretion system (Lackner, Moebius, and
532 Hertweck 2011), and the presence of a specific O-antigen in the lipopolysaccharides, LPS, that
533 make up the outer membrane of these Gram-negative bacteria (Leone et al. 2010). It is not
534 affected, however, by the structural changes in the exopolysaccharide, EPS, secreted matrix
535 (Uzum et al. 2015).

Even though some of the features displayed by the *Rhizopus-Burkholderia* symbiosis are typical for a mutualism, the *Burkholderia* endobacteria appear to be facultative endosymbionts, capable of living both inside and outside eukaryotic cells, a lifestyle similar to that of pathogenic *Legionella*, *Salmonella*, or *Bartonella*. This duality, combined with the ease of experimental manipulation, propelled the *Rhizopus-Burkholderia* symbiosis to become a model for studying the evolution of heritable symbioses. In particular, addressing questions concerning its evolutionary origins, whether it started with the partners interacting as antagonists (Fig. 2), and whether it has already achieved evolutionary stability (Fig. 3) will be a source of rich insights not only into the genetic mechanisms of symbiont vertical transmission but also into other facets of partner coevolution.

V. Heritable Symbiotic Interactions

A. Introduction

As discussed in the preceding sections, symbiont vertical transmission is a principal factor contributing to both the establishment and stability of mutualisms. Importantly, vertical transmission is not exclusive to mutualisms; it can also occur in antagonistic interactions. Vertical transmission can be strict or mixed. In strict vertical transmission symbionts are transferred from a parent exclusively to offspring. In mixed transmission, in addition to being passaged between generations, symbionts move horizontally between members of the same generation. Symbioses with strict vertical transmission are characterized by congruity of partner phylogenetic histories, consistent with partner codiversification (Page 2003). In symbioses with mixed transmission, the extent of horizontal transmission determines the degree of incongruity between partner phylogenies. Interestingly, strict vertical transmission of symbionts tends to be

559 associated with reciprocally obligate partner dependence, whereas mixed transmission is found
560 in associations in which either one or both partners are facultatively dependent on the symbiosis
561 (Fig. 3).

562 Importantly, while in fungi all known heritable associations involve endobacteria that
563 reside inside fungal cells, not all associations formed by fungi with endobacteria are known to be
564 heritable. In heritable symbioses, bacteria are either facultatively or obligately dependent on the
565 fungus. The *Burkholderia* symbionts of *Rhizopus*, discussed in the previous section, as well as
566 *Rhizobium radiobacter* in the root-colonizing *Piriformospora indica* (Sharma et al. 2008)
567 represent facultative heritable endobacteria. In contrast, obligate heritable endosymbionts
568 include two groups of bacteria associated with AMF, *Ca. Glomeribacter gigasporarum*
569 (Bianciotto et al. 2003) and the mycoplasma-related endobacteria, MRE (Naumann, Schüßler,
570 and Bonfante 2010). It is unclear whether the unnamed heritable endosymbiont of *Mortierella*
571 *elongata* (Sato et al. 2010) is a facultative or obligate endobacterium. Remarkably, we are not
572 aware of heritable fungal-bacterial symbioses in which the interacting partners are obligately
573 dependent on each other. Such associations are common in insects, which depend on
574 endobacteria for provision of essential nutrients (McCutcheon and Moran 2012). It remains to
575 be investigated whether this knowledge gap represents a true dearth of reciprocally obligate
576 fungal-bacterial interactions or a detection bias. Recent accumulation of newly discovered
577 associations that involve non-heritable endobacteria suggests that the latter might be the case.
578 Such non-heritable associations include, among others, *Helicobacter pylori* in *Candida albicans*
579 (Siavoshi and Saniee 2014), *Nostoc punctiforme* in *Geosiphon pyriforme* (Schüßler et al. 1994),
580 *Bacillus* spp. in *Ustilago maydis* (Ruiz-Herrera et al. 2015), α -proteobacteria in the
581 ectomycorrhizal fungus *Laccaria bicolor* (Bertaux et al. 2005; Bertaux et al. 2003), and diverse

582 bacteria that inhabit hyphae of phylogenetically diverse fungal endophytes of plants (Hoffman
583 and Arnold 2010). Due to the lack of sufficient data from other systems, our discussion in the
584 following two sections will focus on *Ca. Glomeribacter gigasporarum* and MRE associated with
585 AMF.

586

587 **B. Heritable facultative mutualisms**

588 *Ca. Glomeribacter gigasporarum*, referred hereafter as *Glomeribacter*, is a stable, and
589 structurally integrated endosymbiont found in many representatives of the AMF family
590 Gigasporaceae (Bianciotto, Bandi, et al. 1996; Bianciotto et al. 2003; Mondo et al. 2012). It
591 thrives inside the fungal cells along the different stages of the fungal life cycle, always located
592 inside a compartment structurally resembling a fungal vacuole (Bianciotto, Minerdi, et al. 1996).
593 On the fungal side, the Gigasporaceae, like other AMF, form symbiotic associations with roots
594 of many plants, and may proliferate also in the absence of the endobacteria (Lumini et al. 2007),
595 giving rise to an association that is obligate for the bacterial partner and facultative for the fungal
596 host. A similar disparity is true for all AMF, as they fully depend on their host plants for energy,
597 while plants may complete their life cycle in the absence of AMF.

598 While biodiversity studies have demonstrated that *Glomeribacter* is widespread, they
599 have not identified factors responsible for the evolutionary stability of the Gigasporaceae-
600 *Glomeribacter* symbiosis, which dates back to the early Devonian (Mondo et al. 2012). The
601 *Glomeribacter* genome sequencing revealed that this endobacterium has a reduced genome of
602 1.7 Mb (Ghignone et al. 2012), consistent with its uncultivable status (Jargeat et al. 2004). It
603 lacks metabolic pathways leading to important amino acids, but has many amino acid permeases
604 for uptake of nutrients from the fungus, as expected of an endobacterium that depends on its host

605 for nutrients and energy (Fig. 4). Interestingly, the whole operon for biosynthesis of vitamin B12
606 is present in the *Glomeribacter* genome, but it is not clear whether this might represent any
607 benefit for the fungus. In contrast to animals, which use B12-dependent enzymes for methionine
608 synthesis and methylmalonate metabolism, fungi and land plants rely on B12-independent
609 enzymes for these pathways (Young, Comas, and de Carvalho 2015). Consistent with this
610 expectation, the genome of a model AMF, *Rhizophagus irregularis*, encodes B12-independent
611 enzymes (Tisserant et al. 2013).

612 While the significance of *Glomeribacter* to the AMF hosts could not be gleaned from its
613 genomic sequence, the availability of a stable endosymbiont-free AMF *Gigaspora margarita*
614 BEG34 line, designated as B(-), allowed for direct comparisons with the line containing the
615 endobacterium, B(+). These comparisons revealed several differences, both phenotypic (Lumini
616 et al. 2007) and transcriptional (Salvioli et al. 2016), that speak to the role of *Glomeribacter* in
617 the AMF host. For example, the B(-) AMF line was able to colonize its plant host but was
618 impaired in mycelial growth and spore production compared to the B(+) line (Lumini et al.
619 2007). Moreover, benefits of the endosymbiont presence appeared to extend to the plant host, as
620 the phosphate measurements in *Lotus japonicus* plants revealed a statistically higher phosphate
621 quantity in the symbiosis established by the B(+) versus the B(-) AMF line (Salvioli et al. 2016).
622 In turn, the transcriptome analysis showed that the endobacterium had a stronger effect on the
623 pre-symbiotic phase of the fungus, supporting earlier phenotypic observations that
624 *Glomeribacter* promotes germ tube extension in the AMF host (Lumini et al. 2007; Salvioli et al.
625 2016). Coupling of transcriptomics with physiological and cell biology approaches
626 demonstrated that the bacterium increases the AMF sporulation success, raises the AMF
627 bioenergetic capacity, increasing ATP production, and elicits mechanisms to detoxify reactive

628 oxygen species (Salvioli et al. 2016). Moreover, application of the TAT (transactivator of
629 transcription) peptide to translocate the bioluminescent calcium reporter aequorin revealed that
630 the B(+) AMF line had a lower basal intracellular calcium concentration than the B(–) line,
631 indicating that the endobacterium affects a large number of fungal cell functions, including
632 calcium metabolism, consistent with a potential role as a storage compartment for intracellular
633 calcium. Finally, the fungal mitochondrion and its main metabolic pathways (ATP synthesis,
634 respiration) appear to be important targets of the bacterial presence. Interestingly, the AMF
635 mitochondria are also the first target of strigolactones, the plant hormones that play a key role in
636 plant-fungal signaling (Al-Babili and Bouwmeester 2015; Bonfante and Genre 2015). In the
637 experiments where the B(+) and B(–) AMF lines were treated with a synthetic strigolactone,
638 GR24, the bacteria seemed to react to strigolactones, in agreement with data demonstrating the
639 GR24 treatment induces bacterial cell division (Anca et al. 2009). All these experiments,
640 confirmed by an extensive proteomic analysis (Vannini et al. 2016), revealed that the bacterium,
641 directly or indirectly, affects the oxidative status of the fungus. Moreover, these benefits appear
642 to be transmitted to the host plants (Vannini et al. 2016).

643 Collectively, although *Glomeribacter* exacts a nutritional cost on the AMF, the symbiosis
644 appears to improve the fungal fitness by priming mitochondrial metabolic pathways and
645 provisioning AMF with the tools to face environmental stresses. These observations suggest that
646 evolutionary stability of the Gigasporaceae-*Glomeribacter* mutualism could be best explained by
647 the PFF model (Bull and Rice 1991; Sachs et al. 2004; Weyl et al. 2010), as, at present, there are
648 no indications that non-cooperative partners are sanctioned in this system, a pattern expected
649 under the IPD model (Axelrod and Hamilton 1981; Doebeli and Knowlton 1998; Sachs et al.
650 2004). Neither there is evidence for byproduct cooperation (Connor 1986; Sachs et al. 2004) or

651 compensatory evolution/addiction (Aanen and Hoekstra 2007).

652 Despite the remarkable progress made recently in understanding the Gigasporaceae-
653 *Glomeribacter* symbiosis, there are many outstanding questions. For example, it remains unclear
654 what factors keep this association from evolving towards reciprocally obligate partner
655 dependence predicted by evolutionary theory (Fig. 3). It could be speculated that the benefits to
656 the AMF host depend on the environmental context and the association may break up when the
657 cost of supporting the endosymbiont becomes prohibitive. This scenario would explain why the
658 endobacteria in the Gigasporaceae-*Glomeribacter* symbiosis appear to retain the potential to
659 transmit horizontally and exchange genes, attributes that may have contributed to their
660 evolutionary longevity (Mondo et al. 2012).

661

662 C. Heritable antagonisms

663 The symbiosis between AMF and MRE (mycoplasma related endobacteria) represents an
664 outstanding deviation from the molecular evolution patterns both expected by evolutionary
665 models and detected thus far in heritable endobacteria (McCutcheon and Moran 2012), including
666 *Glomeribacter* (Mondo et al. 2012). In particular, MRE display extraordinary intra-host
667 diversity of their 16S rRNA gene (Naumann, Schübler, and Bonfante 2010; Desirò et al. 2014;
668 Desirò et al. 2015; Toomer et al. 2015) and genomic sequences (Naito, Morton, and Pawlowska
669 2015; Torres-Cortés et al. 2015). In part, this diversity could be attributed to a high mutation
670 rate, related to the loss of DNA repair machinery from the MRE genomes, combined with the
671 apparent activity of mechanisms contributing to genome plasticity, such as recombination
672 machinery and mobile genetic elements (Naito, Morton, and Pawlowska 2015; Naito and
673 Pawlowska 2016). While the mechanisms responsible for genome plasticity are not expected to

674 operate in heritable mutualists with strict vertical transmission, they have been detected in
675 mutualists with mixed transmission (McCutcheon and Moran 2012), including *Glomeribacter*
676 (Mondo et al. 2012). Notably, though, the extent of intra-host diversity displayed by MRE
677 exceeds vastly the diversity exhibited by mutualists with mixed transmission (Naito and
678 Pawlowska 2016). In fact, the co-occurrence of MRE and *Glomeribacter* in several AMF allowed
679 for direct comparisons of their rRNA gene diversity revealing that, while MRE sequences
680 formed highly divergent sequence clusters, no diversity was apparent in *Glomeribacter* (Desirò
681 et al. 2014; Toomer et al. 2015). This disparity in molecular evolution patterns between MRE
682 and heritable mutualists with mixed transmission lead to the hypothesis that MRE may be
683 parasites of AMF (Toomer et al. 2015). This hypothesis is built on the predictions of
684 evolutionary models (Frank 1994, 1996, 1996) suggesting that hosts are expected to benefit from
685 reduced mixing of endosymbiont lineages because genetically uniform endosymbionts are less
686 likely to engage in competition that damages the host (Fig. 5). Bottlenecks imposed by vertical
687 transmission on symbiont populations reduce symbiont diversity inside host individuals, and
688 thus, vertical transmission is expected to limit destructive competition among symbionts for the
689 host resources. On the other hand, decline in symbiont relatedness within a host is predicted to
690 increase host exploitation and favor symbionts that are able to transmit horizontally to secure
691 new hosts.

692 While ascertaining whether MRE are antagonists or mutualists of AMF requires
693 empirical data, inferences about factors that contribute to evolutionary stability of the MRE
694 association with AMF can be made from the molecular evolution patterns evident in their
695 genomes. Given the high mutation rate apparent in MRE, it could be expected that they are
696 vulnerable to genomic degeneration and extinction (McCutcheon and Moran 2012). Yet, co-

697 divergence patterns between MRE and the two fungal lineages in which MRE occur, AMF and
698 the *Endogone* lineage of Mucoromycotina, suggest that the AMF-MRE association may predate
699 the divergence between these two lineages and thus be as old or older than the Gigasporaceae-
700 *Glomeribacter* symbiosis (Desirò et al. 2015; Toomer et al. 2015). It has been postulated that the
701 key factors that prevent MRE from extinction are the mechanisms responsible for genome
702 plasticity in MRE, including the recombination machinery and mobile genetic elements, (Naito,
703 Morton, and Pawlowska 2015; Naito and Pawlowska 2016). Despite these advances, MRE
704 remain an elusive group of endobacteria. Not only their role in the AMF host biology but also
705 the mechanisms of putative horizontal transmission require experimental evaluation.

706

707 **VI. Future Developments**

708 **A. Introduction**

709 The establishment and outcomes of the fungal bacterial interactions are most probably a result of
710 chemical communication where a compound from one partner elicits a response with another
711 compound from the other partner (Baruch et al. 2014; Piispanen and Hogan 2008; Xu et al. 2008;
712 Badri et al. 2009; Nazir et al. 2010; Schroeckh et al. 2009; Sengupta, Chattopadhyay, and
713 Grossart 2013). This is typical for “ping-pong” type communications, where a communication
714 from one interacting partner draws a response from the other partner (Griffin 2012). The correct
715 order of events in ping-pong communication, rather than unique metabolites, could be selective
716 and instrumental in establishing the relationship (like a combinatorial lock). With the advent of
717 modern omics, these ping-pong events could be studied using transcriptomics (Mela et al. 2011;
718 Gkarmiri et al. 2015; Neupane et al. 2015; Mathioni et al. 2013), proteomics (Moretti et al.
719 2010), and aided with metabolomics, allowing for hourly resolution of events during the

establishment of the interaction. Although for multispecies bacterial communities colonizing fungal hyphae this type of study is a major challenge, it would be possible to perform (Moretti et al. 2012) and allow to test predictions of a theoretical model suggesting that complex microbial communities could be stabilized by counteraction of antibiotic synthesis and degradation conducted by different members of the community (Kelsic et al. 2015).

B. Novel tools to study fungal-bacterial metaorganisms

Recently developed technologies, like laser dissection and imaging mass spectrometry (IMS), could be adapted to sample and analyze fungal-bacterial interaction at the microscopic level. Laser dissection could be used to sample single bacterial cells or fungal nuclei from different locations, and combined with single cell genomics/transcriptomics (Kang et al. 2015; Saliba et al. 2014; Teichert et al. 2012), reveal site-dependent activities of various bacteria. IMS (Watrous, Alexandrov, and Dorrestein 2011) has been used to visualize the distribution of selected chemicals such as non-ribosomal antifungal peptides produced in interactions between fungi and bacteria (Michelsen et al. 2015). However, isolating natural fungal-bacterial partners is not trivial and there is a need for new techniques, especially for isolating bacteria from fungal surfaces. Some have already been developed and used to isolate bacteria from fungal highways (Simon et al. 2015) or from floating mycelia (Cuong et al. 2011). Another challenge is to grow natural fungal metaorganisms, since maintaining them on standard rich lab-media could interfere with and break up the association, a problem also faced in highly context-specific lichen metaorganisms (Verma and Behera 2015).

C. Physiological processes known from other host-symbiont systems

743 In this section, we list a few physiological processes known from other host-microbe systems
744 that are also likely to be involved in fungal-bacterial interactions.

745 **Extracellular vesicle trafficking:** All organisms can produce extracellular vesicles (Deatherage
746 and Cookson 2012). In fungal pathogens of humans, these exosomes are important in
747 interactions with the host (Rodrigues et al. 2014), whereas in bacteria they play a role in biofilm
748 communication between cells (Remis et al. 2014; Kulp and Kuehn 2010) and interaction with
749 other bacteria (Kulp and Kuehn 2010; Vasilyeva et al. 2013).

750 **Transfer of interfering RNA:** Extracellular vesicles have been shown to sometimes carry small
751 RNA (Samuel et al. 2015) or DNA (Kulp and Kuehn 2010), which opens up possibilities for
752 interfering with partner organisms (Nicolás and Ruiz-Vázquez 2013).

753 **Unconventional secretion:** Fungi, like all eukaryotes, secrete proteins mainly through the ER-
754 Golgi pathway using N-terminal signal peptides to guide the proteins into the pathway. Proteins
755 without signal peptides can also be secreted through unconventional secretion pathways (Zhang
756 and Schekman 2013). These pathways are important during interaction between host and
757 microorganisms in both plant and animal systems (Ding, Robinson, and Jiang 2014; Öhman et al.
758 2014) and additionally also involved in the production of extracellular vesicles (Zhang and
759 Schekman 2013).

760 **Priming of responses against pathogens by beneficial organisms:** Beneficial bacteria are
761 recognized by similar systems as pathogens and can induce enhanced immune functions against
762 later attacks by pathogens, thus priming the defenses. Such priming responses are a hot topic in
763 both plant and animal systems (Chu and Mazmanian 2013; Conrath 2009; Aranega-Bou et al.
764 2014; Val et al. 2008) and can be expected to be important for both non-heritable and heritable
765 fungal bacterial interactions.

766

767 **VII. Closing Remarks**

768 The recent explosion of newly discovered fungal-bacterial interactions suggests that they are
769 more common and important than previously thought. In addition to their significance in
770 ecosystem functioning, many fungal-bacterial associations are central to human health,
771 agriculture, forestry, and bioremediation. While some of these important symbioses are already
772 in the forefront of data gathering and interpretation, many still remain unknown because of the
773 microscopic scale of the interacting partners, the complexity of their communities, and the
774 intricate nature of the relations that connect them. The advent and expansion of new techniques,
775 which allow for exploration and characterization of microbiota in natural and man-made habitats,
776 carries a promise that these obscure systems will soon be discovered and understood at the level
777 achieved for macroorganisms and their interactions. Here, we hope that our discussion will
778 inspire both fungal biologists and prokaryotic microbiologists to develop cross-disciplinary
779 approaches allowing for discovery and characterization of novel links between fungi and
780 bacteria. Until microbiota-specific conceptual tools are established, these explorations could be
781 guided by ecological and evolutionary frameworks that already exist for interspecific interactions
782 among macroorganisms. Collectively, a combination of the omics approaches, genetic
783 experiments, and ecological and evolutionary tools will allow us to expand the knowledge of
784 fungal-bacterial biodiversity and understand the mechanisms underlying these inter-domain
785 interactions.

786

787 **Acknowledgements**

788 We thank Olga Lastovetsky for comments on the manuscript. This work was supported by the

789 National Science Foundation grant IOS-1261004 to TEP and the Torino University 60% grant to
790 PB.

791

792 **References**

793 Aanen, D. K., and T. Bisseling. 2014. Microbiology. The birth of cooperation. *Science* 345

794 (6192):29-30.

795 Aanen, D. K., and R. F. Hoekstra. 2007. The evolution of obligate mutualism: if you can't beat

796 'em, join 'em. *Trends in Ecology & Evolution* 22 (10):506-509.

797 Al-Babili, S., and H. J. Bouwmeester. 2015. Strigolactones, a novel carotenoid-derived plant

798 hormone. *Annual Review of Plant Biology* (66):161-186.

799 Alvarez, F. J., L. M. Douglas, and J. B. Konopka. 2007. Sterol-rich plasma membrane domains

800 in fungi. *Eukaryotic Cell* 6 (5):755-763.

801 Anca, I. A., E. Lumini, S. Ghignone, A. Salvioli, V. Bianciotto, and P. Bonfante. 2009. The *ftsZ*

802 gene of the endocellular bacterium '*Candidatus Glomeribacter gigasporarum*' is

803 preferentially expressed during the symbiotic phases of its host mycorrhizal fungus.

804 *Molecular Plant-Microbe Interactions* 22 (3):302-10.

805 Angebault, C., F. Djossou, S. Abelanet, E. Permal, M. Ben Soltana, L. Diancourt, C. Bouchier,

806 P.-L. Woerther, F. Catzefflis, A. Andremont, C. d'Enfert, and M.-E. Bournoux. 2013.

807 *Candida albicans* is not always the preferential yeast colonizing humans: A study in

808 Wayampi Amerindians. *Journal of Infectious Diseases* 208 (10):1705-1716.

809 Aranega-Bou, P., M. D. Leyva, I. Finiti, P. García-Agustín, and C. González-Bosch. 2014.

810 Priming of plant resistance by natural compounds. Hexanoic acid as a model. *Frontiers in*

811 *Plant Science* 5:488.

812 Archibald, F. 1983. *Lactobacillus plantarum*, an organism not requiring iron. *FEMS*

813 *Microbiology Letters* 19 (1):29-32.

814 Artis, D. 2008. Epithelial-cell recognition of commensal bacteria and maintenance of immune
815 homeostasis in the gut. *Nature Reviews Immunology* 8 (6):411-420.

816 Ausubel, F. M. 2005. Are innate immune signaling pathways in plants and animals conserved?
817 *Nature Immunology* 6 (10):973-979.

818 Axelrod, R., and W. D. Hamilton. 1981. The evolution of cooperation. *Science* 211 (4489):1390-
819 1396.

820 Badri, D. V., T. L. Weir, D. van der Lelie, and J. M. Vivanco. 2009. Rhizosphere chemical
821 dialogues: plant-microbe interactions. *Current Opinion in Biotechnology* 20 (6):642-650.

822 Banitz, T., K. Johst, L. Y. Wick, S. Schamfuss, H. Harms, and K. Frank. 2014. Highways versus
823 pipelines: contributions of two fungal transport mechanisms to efficient bioremediation.
824 *Environmental Microbiology Reports* 5 (4):211-218.

825 Barron, G. L. 1988. Microcolonies of bacteria as a nutrient source for lignicolous and other
826 fungi. *Canadian Journal of Botany* 66 (12):2505-2510.

827 Barron, G. L. 2003. Predatory fungi, wood decay, and the carbon cycle. *Biodiversity* 4 (1):3-9.

828 Baruch, M., I. Belotserkovsky, B. B. Hertzog, M. Ravins, E. Dov, K. S. McIver, Y. S. Le Breton,
829 Y. Zhou, C. Y. Cheng, and E. Hanski. 2014. An extracellular bacterial pathogen
830 modulates host metabolism to regulate its own sensing and proliferation. *Cell* 156 (1-
831 2):97-108.

832 Baschien, C., G. Rode, U. Boeckelmann, P. Goetz, and U. Szewzyk. 2009. Interactions between
833 hyphosphere-associated bacteria and the fungus *Cladosporium herbarum* on aquatic leaf
834 litter. *Microbial Ecology* 58 (3):642-650.

835 Bascompte, J., and P. Jordano. 2013. *Mutualistic Networks*. Princeton: Princeton University
836 Press.

837 Beneduzi, A., A. Ambrosini, and L. M. P. Passaglia. 2012. Plant growth-promoting rhizobacteria
838 (PGPR): Their potential as antagonists and biocontrol agents. *Genetics and Molecular*
839 *Biology* 35 (4 Suppl):1044-1051.

840 Berman, J., and P. E. Sudbery. 2002. *Candida albicans*: a molecular revolution built on lessons
841 from budding yeast. *Nature Reviews Genetics* 3 (12):918-30.

842 Bertaux, J., M. Schmid, P. Hutzler, A. Hartmann, J. Garbaye, and P. Frey-Klett. 2005.
843 Occurrence and distribution of endobacteria in the plant-associated mycelium of the
844 ectomycorrhizal fungus *Laccaria bicolor* S238N. *Environmental Microbiology* 7
845 (11):1786-1795.

846 Bertaux, J., M. Schmid, N. C. Prevost-Boure, J. L. Churin, A. Hartmann, J. Garbaye, and P.
847 Frey-Klett. 2003. *In situ* identification of intracellular bacteria related to *Paenibacillus*
848 spp. in the mycelium of the ectomycorrhizal fungus *Laccaria bicolor* S238N. *Applied*
849 *and Environmental Microbiology* 69 (7):4243-4248.

850 Biagi, E., M. Candela, S. Fairweather-Tait, C. Franceschi, and P. Brigidi. 2012. Ageing of the
851 human metaorganism: the microbial counterpart. *Age* 34 (1):247-267.

852 Bianciotto, V., S. Andreotti, R. Balestrini, P. Bonfante, and S. Perotto. 2001. Mucoid mutants of
853 the biocontrol strain *Pseudomonas fluorescens* CHA0 show increased ability in biofilm
854 formation on mycorrhizal and nonmycorrhizal carrot roots. *Molecular Plant-Microbe*
855 *Interactions* 14 (2):255-60.

856 Bianciotto, V., C. Bandi, D. Minerdi, M. Sironi, H. V. Tichy, and P. Bonfante. 1996. An
857 obligately endosymbiotic mycorrhizal fungus itself harbors obligately intracellular
858 bacteria. *Applied and Environmental Microbiology* 62 (8):3005-3010.

859 Bianciotto, V., E. Lumini, P. Bonfante, and P. Vandamme. 2003. '*Candidatus* Glomeribacter
860 gigasporarum' gen. nov., sp nov., an endosymbiont of arbuscular mycorrhizal fungi.
861 *International Journal of Systematic and Evolutionary Microbiology* 53:121-124.

862 Bianciotto, V., D. Minerdi, S. Perotto, and P. Bonfante. 1996. Cellular interactions between
863 arbuscular mycorrhizal fungi and rhizosphere bacteria. *Protoplasma* 193 (1-4):123-131.

864 Bonfante, P., and I. A. Anca. 2009. Plants, mycorrhizal fungi, and bacteria: a network of
865 interactions. *Annual Review of Microbiology* 63:363-83.

866 Bonfante, P., and A. Genre. 2015. Arbuscular mycorrhizal dialogues: do you speak 'plantish' or
867 'fungish'? *Trends Plant Sci* 20 (3):150-4.

868 Boon, C., Y. Deng, L. H. Wang, Y. He, J. L. Xu, Y. Fan, S. Q. Pan, and L. H. Zhang. 2008. A
869 novel DSF-like signal from *Burkholderia cenocepacia* interferes with *Candida albicans*
870 morphological transition. *ISME Journal* 2 (1):27-36.

871 Bosch, T. C. G., and M. J. McFall-Ngai. 2011. Metaorganisms as the new frontier. *Zoology* 114
872 (4):185-190.

873 Bravo, D., G. Cailleau, S. Bindschedler, A. Simon, D. Job, E. Verrecchia, and P. Junier. 2013.
874 Isolation of oxalotrophic bacteria able to disperse on fungal mycelium. *FEMS*
875 *Microbiology Letters* 348 (2):157-166.

876 Broadbent, D. 1966. Antibiotics produced by fungi. *Botanical Review* 32 (3):219-242.

877 Brown, S. D., S. M. Utturkar, D. M. Klingeman, C. M. Johnson, S. L. Martin, M. L. Land, T. Y.
878 S. Lu, C. W. Schadt, M. J. Doktycz, and D. A. Pelletier. 2012. Twenty-one genome
879 sequences from *Pseudomonas* species and 19 genome sequences from diverse bacteria
880 isolated from the rhizosphere and endosphere of *Populus deltoides*. *Journal of*
881 *Bacteriology* 194 (21):5991-5993.

882 Bull, J. J., and W. R. Rice. 1991. Distinguishing mechanisms for the evolution of cooperation.
883 *Journal of Theoretical Biology* 149 (1):63-74.

884 Chu, H. T., and S. K. Mazmanian. 2013. Innate immune recognition of the microbiota promotes
885 host-microbial symbiosis. *Nature Immunology* 14 (7):668-675.

886 Connor, R. C. 1986. Pseudo-reciprocity: investing in mutualism. *Animal Behaviour* 34:1562–
887 1584.

888 Conrath, U. 2009. Priming of induced plant defense responses. *Plant Innate Immunity* 51:361-
889 395.

890 Cugini, C., M. W. Calfee, J. M. Farrow, 3rd, D. K. Morales, E. C. Pesci, and D. A. Hogan. 2007.
891 Farnesol, a common sesquiterpene, inhibits PQS production in *Pseudomonas aeruginosa*.
892 *Molecular Microbiology* 65 (4):896-906.

893 Cuong, N. D., M. H. Nicolaisen, J. Sørensen, and S. Olsson. 2011. Hyphae-colonizing
894 *Burkholderia* sp.—A new source of biological control agents against sheath blight
895 disease (*Rhizoctonia solani* AG1-IA) in rice. *Microbial Ecology* 62 (2):425-434.

896 Davis-Hanna, A., A. E. Piispanen, L. I. Stateva, and D. A. Hogan. 2008. Farnesol and dodecanol
897 effects on the *Candida albicans* Ras1-cAMP signalling pathway and the regulation of
898 morphogenesis. *Molecular Microbiology* 67 (1):47-62.

899 de Boer, W., L. B. Folman, R. C. Summerbell, and L. Boddy. 2005. Living in a fungal world:
900 impact of fungi on soil bacterial niche development. *FEMS Microbiology Reviews* 29
901 (4):795-811.

902 de Boer, W., and A. van der Wal. 2008. Interactions between saprotrophic basidiomycetes and
903 bacteria. In *Ecology of Saprotrophic Basidiomycetes*, edited by L. Boddy, J. C. Frankland
904 and P. van West. San Diego, CA: Elsevier Academic Press Inc.

905 Deatherage, B. L., and B. T. Cookson. 2012. Membrane vesicle release in bacteria, eukaryotes,
906 and archaea: a conserved yet underappreciated aspect of microbial life. *Infection and*
907 *Immunity* 80 (6):1948-1957.

908 Desirò, A., A. Faccio, A. Kaech, M. I. Bidartondo, and P. Bonfante. 2015. *Endogone*, one of the
909 oldest plant-associated fungi, host unique Mollicutes-related endobacteria. *New*
910 *Phytologist* 205 (4):1464-1472.

911 Desirò, A., A. Salvioli, E. L. Ngonkeu, S. J. Mondo, S. Epis, A. Faccio, A. Kaech, T. E.
912 Pawlowska, and P. Bonfante. 2014. Detection of a novel intracellular microbiome hosted
913 in arbuscular mycorrhizal fungi. *ISME Journal* 8 (2):257–270.

914 Deveau, A., C. Brulé, B. Palin, D. Champmartin, P. Rubini, J. Garbaye, A. Sarniguet, and P.
915 Frey-Klett. 2010. Role of fungal trehalose and bacterial thiamine in the improved survival
916 and growth of the ectomycorrhizal fungus *Laccaria bicolor* S238N and the helper
917 bacterium *Pseudomonas fluorescens* BBc6R8. *Environmental Microbiology Reports* 2
918 (4):560-568.

919 Deveau, A., H. Gross, E. Morin, T. Karpinets, S. Utturkar, S. Mehnaz, F. Martin, P. Frey-Klett,
920 and J. Labbé. 2014. Genome sequence of the mycorrhizal helper bacterium *Pseudomonas*
921 *fluorescens* BBc6R8. *Genome Announcements* 2 (1):e01152-13.

922 Ding, Y., D. G. Robinson, and L. W. Jiang. 2014. Unconventional protein secretion (UPS)
923 pathways in plants. *Current Opinion in Cell Biology* 29:107-115.

924 Doebeli, M., and N. Knowlton. 1998. The evolution of interspecific mutualisms. *Proceedings of*
925 *the National Academy of Sciences of the United States of America* 95 (15):8676-8680.

926 Eggimann, P., J. Garbino, and D. Pittet. 2003. Epidemiology of *Candida* species infections in
927 critically ill non-immunosuppressed patients. *Lancet Infectious Diseases* 3 (11):685-702.

928 Espuny Tomas, J. M., D. M. Simon-Pujol, F. Congregado, and G. Suarez Fernandez. 1982.
929 Nature of antagonism of fungi by bacteria isolated from soils. *Soil Biology &*
930 *Biochemistry* 14 (6):557-560.

931 Frank, S. A. 1994. Kin selection and virulence in the evolution of protocells and parasites.
932 *Proceedings of the Royal Society of London Series B-Biological Sciences* 258
933 (1352):153-161.

934 Frank, S. A. 1996. Host-symbiont conflict over the mixing of symbiotic lineages. *Proceedings of*
935 *the Royal Society of London Series B-Biological Sciences* 263 (1368):339-344.

936 Frank, S. A. 1996. Models of parasite virulence. *Quarterly Review of Biology* 71 (1):37-78.

937 Frey-Klett, P., P. Burlinson, A. Deveau, M. Barret, M. Tarkka, and A. Sarniguet. 2011.
938 Bacterial-fungal interactions: hyphens between agricultural, clinical, environmental, and
939 food microbiologists. *Microbiology and Molecular Biology Reviews* 75 (4):583-609.

940 Fujimura, R., A. Nishimura, S. Ohshima, Y. Sato, T. Nishizawa, K. Oshima, M. Hattori, K.
941 Narisawa, and H. Ohta. 2014. Draft genome sequence of the betaproteobacterial
942 endosymbiont associated with the fungus *Mortierella elongata* FMR23-6. *Genome*
943 *Announcements* 2 (6):e01272-14.

944 Furuno, S., K. Pazolt, C. Rabe, T. R. Neu, H. Harms, and L. Y. Wick. 2010. Fungal mycelia
945 allow chemotactic dispersal of polycyclic aromatic hydrocarbon-degrading bacteria in
946 water-unsaturated systems. *Environmental Microbiology* 12 (6):1391-1398.

947 Ganz, T. 2009. Iron in innate immunity: starve the invaders. *Current Opinion in Immunology* 21
948 (1):63-67.

949 Garbaye, J. 1994. Helper bacteria: a new dimension to the mycorrhizal symbiosis. *New*
950 *Phytologist* 128:197-210.

951 Ghignone, S., A. Salvioli, I. Anca, E. Lumini, G. Ortu, L. Petiti, S. Cruveiller, V. Bianciotto, P.
 952 Piffanelli, L. Lanfranco, and P. Bonfante. 2012. The genome of the obligate
 953 endobacterium of an AM fungus reveals an interphylum network of nutritional
 954 interactions. *ISME Journal* 6 (1):136-145.
 955 Gibson, J., A. Sood, and D. A. Hogan. 2009. *Pseudomonas aeruginosa-Candida albicans*
 956 interactions: localization and fungal toxicity of a phenazine derivative. *Applied and*
 957 *Environmental Microbiology* 75 (2):504-513.
 958 Gkarmiri, K., R. D. Finlay, S. Alström, E. Thomas, M. A. Cubeta, and N. Högborg. 2015.
 959 Transcriptomic changes in the plant pathogenic fungus *Rhizoctonia solani* AG-3 in
 960 response to the antagonistic bacteria *Serratia proteamaculans* and *Serratia plymuthica*.
 961 *BMC Genomics* 16:630.
 962 Gomulkiewicz, R., D. M. Drown, M. F. Dybdahl, W. Godsoe, S. L. Nuismer, K. M. Pepin, B. J.
 963 Ridenhour, C. I. Smith, and J. B. Yoder. 2007. Dos and don'ts of testing the geographic
 964 mosaic theory of coevolution. *Heredity* 98 (5):249-258.
 965 Griffin, E. A. 2012. *A first look at communication theory*. 8th ed. ed. New York: McGraw-Hill.
 966 Grube, M., and G. Berg. 2009. Microbial consortia of bacteria and fungi with focus on the lichen
 967 symbiosis. *Fungal Biology Reviews* 23 (3):72-85.
 968 Gupta, N., A. Haque, G. Mukhopadhyay, R. P. Narayan, and R. Prasad. 2005. Interactions
 969 between bacteria and *Candida* in the burn wound. *Burns* 31 (3):375-8.
 970 Hall, R. A., K. J. Turner, J. Chaloupka, F. Cottier, L. De Sordi, D. Sanglard, L. R. Levin, J.
 971 Buck, and F. A. Mühlschlegel. 2011. The quorum-sensing molecules farnesol/homoserine
 972 lactone and dodecanol operate via distinct modes of action in *Candida albicans*.
 973 *Eukaryotic Cell* 10 (8):1034-42.

974 Hartmann, A., M. Schmid, D. van Tuinen, and G. Berg. 2009. Plant-driven selection of
 975 microbes. *Plant and Soil* 321 (1-2):235-257.

976 Herre, E. A., N. Knowlton, U. G. Mueller, and S. A. Rehner. 1999. The evolution of mutualisms:
 977 exploring the paths between conflict and cooperation. *Trends in Ecology & Evolution* 14
 978 (2):49-53.

979 Hoffman, M. T., and A. E. Arnold. 2010. Diverse bacteria inhabit living hyphae of
 980 phylogenetically diverse fungal endophytes. *Applied and Environmental Microbiology* 76
 981 (12):4063-4075.

982 Hogan, D. A., and R. Kolter. 2002. *Pseudomonas-Candida* interactions: An ecological role for
 983 virulence factors. *Science* 296 (5576):2229-2232.

984 Hogan, D. A., Å. Vik, and R. Kolter. 2004. A *Pseudomonas aeruginosa* quorum-sensing
 985 molecule influences *Candida albicans* morphology. *Molecular Microbiology* 54
 986 (5):1212-1223.

987 Hom, E. F., and A. W. Murray. 2014. Plant-fungal ecology. Niche engineering demonstrates a
 988 latent capacity for fungal-algal mutualism. *Science* 345 (6192):94-8.

989 Hoppe, B., T. Kahl, P. Karasch, T. Wubet, J. Bauhus, F. Buscot, and D. Kruger. 2014. Network
 990 analysis reveals ecological links between N-fixing bacteria and wood-decaying fungi.
 991 *Plos One* 9 (2):e88141.

992 Hornby, J. M., E. C. Jensen, A. D. Lisec, J. J. Tasto, B. Jahnke, R. Shoemaker, P. Dussault, and
 993 K. W. Nickerson. 2001. Quorum sensing in the dimorphic fungus *Candida albicans* is
 994 mediated by farnesol. *Applied and Environmental Microbiology* 67 (7):2982-92.

995 Hsueh, Y. P., P. Mahanti, F. C. Schroeder, and P. W. Sternberg. 2013. Nematode-trapping fungi
 996 eavesdrop on nematode pheromones. *Current Biology* 23 (1):83-86.

997 Huang, X.-F., J. M. Chaparro, K. F. Reardon, R. Zhang, Q. Shen, and J. M. Vivanco. 2014.
 998 Rhizosphere interactions: root exudates, microbes, and microbial communities. *Botany*
 999 92:267–275.
 1000 Hughes, W., and H. Kim. 1973. Mycoflora in cystic fibrosis: Some ecologic aspects of
 1001 *Pseudomonas aeruginosa* and *Candida albicans*. *Mycopathologia et Mycologia*
 1002 *Applicata* 50 (3):261-269.
 1003 Ipcho, S., T. Sundelin, G. Erbs, H. C. Kistler, M.-A. Newman, and S. Olsson. 2016. Fungal
 1004 innate immunity induced by bacterial Microbe-Associated Molecular Patterns (MAMPs).
 1005 *Submitted*.
 1006 Jansa, J., P. Bukovská, and M. Gryndler. 2013. Mycorrhizal hyphae as ecological niche for
 1007 highly specialized hypersymbionts – or just soil free-riders? *Frontiers in Plant Science*
 1008 4:134.
 1009 Janzen, D. H. 1985. On ecological fitting. *Oikos* 45 (3):308-310.
 1010 Jargeat, P., C. Cosseau, B. Ola'h, A. Jauneau, P. Bonfante, J. Batut, and G. Bécard. 2004.
 1011 Isolation, free-living capacities, and genome structure of "*Candidatus* Glomeribacter
 1012 gigasporarum," the endocellular bacterium of the mycorrhizal fungus *Gigaspora*
 1013 *margarita*. *Journal of Bacteriology* 186 (20):6876-6884.
 1014 Jochum, C. C., L. E. Osborne, and G. Y. Yuen. 2006. *Fusarium* head blight biological control
 1015 with *Lysobacter enzymogenes*. *Biological Control* 39 (3):336-344.
 1016 Kai-Larsen, Y., G. H. Gudmundsson, and B. Agerberth. 2014. A review of the innate immune
 1017 defence of the human foetus and newborn, with the emphasis on antimicrobial peptides.
 1018 *Acta Paediatrica* 103 (10):1000-1008.

1019 Kang, Y., I. McMillan, M. H. Norris, and T. T. Hoang. 2015. Single prokaryotic cell isolation
1020 and total transcript amplification protocol for transcriptomic analysis. *Nature Protocols*
1021 10 (7):974-84.

1022 Kelsic, E. D., J. Zhao, K. Vetsigian, and R. Kishony. 2015. Counteraction of antibiotic
1023 production and degradation stabilizes microbial communities. *Nature* 521 (7553):516.

1024 Kerr, J. 1994. Inhibition of fungal growth by *Pseudomonas aeruginosa* and *Pseudomonas*
1025 *cepacia* isolated from patients with cystic fibrosis. *The Journal of Infection* 28 (3):305-
1026 10.

1027 Kerr, J. R. 1999. Bacterial inhibition of fungal growth and pathogenicity. *Microbial Ecology in*
1028 *Health and Disease* 11 (3):129-142.

1029 Kiers, E. T., M. Duhamel, Y. Beesetty, J. A. Mensah, O. Franken, E. Verbruggen, C. R.
1030 Fellbaum, G. A. Kowalchuk, M. M. Hart, A. Bago, T. M. Palmer, S. A. West, P.
1031 Vandenkoornhuyse, J. Jansa, and H. Bucking. 2011. Reciprocal rewards stabilize
1032 cooperation in the mycorrhizal symbiosis. *Science* 333 (6044):880-882.

1033 Kobayashi, D. Y., and J. A. Crouch. 2009. Bacterial/fungal interactions: From pathogens to
1034 mutualistic endosymbionts. *Annual Review of Phytopathology* 47:63-82.

1035 Kohlmeier, S., T. H. M. Smits, R. M. Ford, C. Keel, H. Harms, and L. Y. Wick. 2005. Taking the
1036 fungal highway: Mobilization of pollutant-degrading bacteria by fungi. *Environmental*
1037 *Science & Technology* 39 (12):4640-4646.

1038 Kulp, A., and M. J. Kuehn. 2010. Biological functions and biogenesis of secreted bacterial outer
1039 membrane vesicles. *Annual Review of Microbiology* 64:163-184.

1040 Labbé, J. L., D. J. Weston, N. Dunkirk, D. A. Pelletier, and G. A. Tuskan. 2014. Newly
 1041 identified helper bacteria stimulate ectomycorrhizal formation in *Populus*. *Frontiers in*
 1042 *Plant Science* 5:579.
 1043 Lackner, G., and C. Hertweck. 2011. Impact of endofungal bacteria on infection biology, food
 1044 safety, and drug development. *PLoS Pathogens* 7 (6):e1002096.
 1045 Lackner, G., N. Moebius, and C. Hertweck. 2011. Endofungal bacterium controls its host by an
 1046 *hrp* type III secretion system. *ISME Journal* 5 (2):252-261.
 1047 Lackner, G., L. P. Partida-Martinez, and C. Hertweck. 2009. Endofungal bacteria as producers of
 1048 mycotoxins. *Trends in Microbiology* 17 (12):570-576.
 1049 Lemanceau, P., D. Expert, F. Gaymard, P. A. H. M. Bakker, and J. F. Briat. 2009. Role of iron in
 1050 plant-microbe interactions. *Advances in Botanical Research* 51:491-549.
 1051 Leone, M. R., G. Lackner, A. Silipo, R. Lanzetta, A. Molinaro, and C. Hertweck. 2010. An
 1052 unusual galactofuranose lipopolysaccharide that ensures the intracellular survival of
 1053 toxin-producing bacteria in their fungal host. *Angewandte Chemie* 49 (41):7476-7480.
 1054 Leveau, J. H. J., and G. M. Preston. 2008. Bacterial mycophagy: definition and diagnosis of a
 1055 unique bacterial-fungal interaction. *New Phytologist* 177 (4):859-876.
 1056 Lewis, D. H. 1985. Symbiosis and mutualism: crisp concepts and soggy semantics. In *The*
 1057 *Biology of Mutualism. Ecology and Evolution.*, edited by D. H. Boucher. New York:
 1058 Oxford University Press.
 1059 Li, S., L. Du, G. Yuen, and S. D. Harris. 2006. Distinct ceramide synthases regulate polarized
 1060 growth in the filamentous fungus *Aspergillus nidulans*. *Molecular Biology of the Cell* 17
 1061 (3):1218-1227.

1062 Li, X., C. S. Quan, H. Y. Yu, and S. D. Fan. 2008. Multiple effects of a novel compound from
1063 *Burkholderia cepacia* against *Candida albicans*. *FEMS Microbiology Letters* 285
1064 (2):250-6.

1065 Lindsay, A. K., A. Deveau, A. E. Piispanen, and D. A. Hogan. 2012. Farnesol and cyclic AMP
1066 signaling effects on the hypha-to-yeast transition in *Candida albicans*. *Eukaryotic Cell* 11
1067 (10):1219-25.

1068 Lister, P. D., D. J. Wolter, and N. D. Hanson. 2009. Antibacterial-resistant *Pseudomonas*
1069 *aeruginosa*: Clinical impact and complex regulation of chromosomally encoded
1070 resistance mechanisms. *Clinical Microbiology Reviews* 22 (4):582-610.

1071 Lopez-Medina, E., D. Fan, L. A. Coughlin, E. X. Ho, I. L. Lamont, C. Reimann, L. V. Hooper,
1072 and A. Y. Koh. 2015. *Candida albicans* inhibits *Pseudomonas aeruginosa* virulence
1073 through suppression of pyochelin and pyoverdine biosynthesis. *PLoS Pathogens* 11
1074 (8):e1005129.

1075 Lott, T. J., R. E. Fundyga, R. J. Kuykendall, and J. Arnold. 2005. The human commensal yeast,
1076 *Candida albicans*, has an ancient origin. *Fungal Genetics and Biology* 42 (5):444-451.

1077 Lumini, E., V. Bianciotto, P. Jargeat, M. Novero, A. Salvioli, A. Faccio, G. Bécard, and P.
1078 Bonfante. 2007. Presymbiotic growth and sporal morphology are affected in the
1079 arbuscular mycorrhizal fungus *Gigaspora margarita* cured of its endobacteria. *Cellular*
1080 *Microbiology* 9 (7):1716-1729.

1081 Lyons, J. I., S. Y. Newell, R. P. Brown, and M. A. Moran. 2005. Screening for bacterial-fungal
1082 associations in a south-eastern US salt marsh using pre-established fungal monocultures.
1083 *FEMS Microbiology Ecology* 54 (2):179-187.

1084 Markel, T. A., P. R. Crisostomo, M. Wang, C. M. Herring, K. K. Meldrum, K. D. Lillemoe, and
 1085 D. R. Meldrum. 2007. The struggle for iron: gastrointestinal microbes modulate the host
 1086 immune response during infection. *Journal of Leukocyte Biology* 81 (2):393-400.
 1087 Martin, B. D., and E. Schwab. 2012. Current usage of symbiosis and associated terminology.
 1088 *International Journal of Biology* 5 (1):32-45.
 1089 Mathioni, S. M., N. Patel, B. Riddick, J. A. Sweigard, K. J. Czymmek, J. L. Caplan, S. G.
 1090 Kunjeti, S. Kunjeti, V. Raman, B. I. Hillman, D. Y. Kobayashi, and N. M. Donofrio.
 1091 2013. Transcriptomics of the rice blast fungus *Magnaporthe oryzae* in response to the
 1092 bacterial antagonist *Lysobacter enzymogenes* reveals candidate fungal defense response
 1093 genes. *PLoS One* 8 (10):e76487.
 1094 McCutcheon, J. P., and N. A. Moran. 2012. Extreme genome reduction in symbiotic bacteria.
 1095 *Nature Reviews Microbiology* 10 (1):13-26.
 1096 McCutcheon, J. P., and C. D. von Dohlen. 2011. An interdependent metabolic patchwork in the
 1097 nested symbiosis of mealybugs. *Current Biology* 21 (16):1366-72.
 1098 McFrederick, Q. S., W. T. Wcislo, D. R. Taylor, H. D. Ishak, S. E. Dowd, and U. G. Mueller.
 1099 2012. Environment or kin: whence do bees obtain acidophilic bacteria? *Molecular*
 1100 *Ecology* 21 (7):1754-1768.
 1101 McManus, B. A., and D. C. Coleman. 2014. Molecular epidemiology, phylogeny and evolution
 1102 of *Candida albicans*. *Infection Genetics and Evolution* 21:166-178.
 1103 Mela, F., K. Fritsche, W. de Boer, J. A. van Veen, L. H. de Graaff, M. van den Berg, and J. H. J.
 1104 Leveau. 2011. Dual transcriptional profiling of a bacterial/fungal confrontation:
 1105 *Collimonas fungivorans* versus *Aspergillus niger*. *ISME Journal* 5 (9):1494-1504.

1106 Mello, A., G. C. Ding, Y. M. Piceno, C. Napoli, L. M. Tom, T. Z. DeSantis, G. L. Andersen, K.
 1107 Smalla, and P. Bonfante. 2013. Truffle brûlés have an impact on the diversity of soil
 1108 bacterial communities. *PLoS One* 8 (4):e61945.
 1109 Michelsen, C. F., J. Watrous, M. A. Glaring, R. Kersten, N. Koyama, P. C. Dorrestein, and P.
 1110 Stougaard. 2015. Nonribosomal peptides, key biocontrol components for *Pseudomonas*
 1111 *fluorescens* In5, isolated from a Greenlandic suppressive soil. *mBio* 6 (2):e00079-15.
 1112 Moebius, N., Z. Üzümlü, J. Dijksterhuis, G. Lackner, and C. Hertweck. 2014. Active invasion of
 1113 bacteria into living fungal cells. *eLife* 3:e03007.
 1114 Mondo, S. J., K. H. Toomer, J. B. Morton, Y. Lekberg, and T. E. Pawlowska. 2012. Evolutionary
 1115 stability in a 400-million-year-old heritable facultative mutualism. *Evolution* 66 (8):2564-
 1116 2576.
 1117 Moran, N. A., J. P. McCutcheon, and A. Nakabachi. 2008. Genomics and evolution of heritable
 1118 bacterial symbionts. *Annual Review of Genetics* 42:165-190.
 1119 Moretti, M., A. Grunau, D. Minerdi, P. Gehrig, B. Roschitzki, L. Eberl, A. Garibaldi, M. L.
 1120 Gullino, and K. Riedel. 2010. A proteomics approach to study synergistic and
 1121 antagonistic interactions of the fungal-bacterial consortium *Fusarium oxysporum* wild-
 1122 type MSA 35. *Proteomics* 10 (18):3292-3320.
 1123 Moretti, M., D. Minerdi, P. Gehrig, A. Garibaldi, M. L. Gullino, and K. Riedel. 2012. A
 1124 bacterial-fungal metaproteomic analysis enlightens an intriguing multicomponent
 1125 interaction in the rhizosphere of *Lactuca sativa*. *Journal of Proteome Research* 11
 1126 (4):2061-2077.
 1127 Naito, M., J. B. Morton, and T. E. Pawlowska. 2015. Minimal genomes of mycoplasma-related
 1128 endobacteria are plastic and contain host-derived genes for sustained life within

1129 Glomeromycota. *Proceedings of the National Academy of Sciences of the United States*
1130 *of America* 112 (25):7791-7796.

1131 Naito, M., and T. E. Pawlowska. 2016. The role of mobile genetic elements in evolutionary
1132 longevity of heritable endobacteria. *Mobile Genetic Elements* 6 (1):e1136375.

1133 Napoli, C., A. Mello, A. Borra, A. Vizzini, P. Sourzat, and P. Bonfante. 2010. *Tuber*
1134 *melanosporum*, when dominant, affects fungal dynamics in truffle grounds. *New*
1135 *Phytologist* 185 (1):237-247.

1136 Naumann, M., A. Schüßler, and P. Bonfante. 2010. The obligate endobacteria of arbuscular
1137 mycorrhizal fungi are ancient heritable components related to the Mollicutes. *ISME*
1138 *Journal* 4 (7):862-871.

1139 Nazir, R., D. I. Tazetdinova, and J. D. van Elsas. 2014. *Burkholderia terrae* BS001 migrates
1140 proficiently with diverse fungal hosts through soil and provides protection from
1141 antifungal agents. *Frontiers in Microbiology* 5:598.

1142 Nazir, R., J. A. Warmink, H. Boersma, and J. D. van Elsas. 2010. Mechanisms that promote
1143 bacterial fitness in fungal-affected soil microhabitats. *FEMS Microbiology Ecology* 71
1144 (2):169-185.

1145 Nazir, R., J. A. Warmink, D. C. Voordes, H. H. van de Bovenkamp, and J. D. van Elsas. 2013.
1146 Inhibition of mushroom formation and induction of glycerol release — Ecological
1147 strategies of *Burkholderia terrae* BS001 to create a hospitable niche at the fungus
1148 *Lyophyllum* sp. strain Karsten. *Microbial Ecology* 65 (1):245-254.

1149 Neupane, S., R. D. Finlay, S. Alström, M. Elfstrand, and N. Högberg. 2015. Transcriptional
1150 responses of the bacterial antagonist *Serratia plymuthica* to the fungal phytopathogen
1151 *Rhizoctonia solani*. *Environmental Microbiology Reports* 7 (1):123-127.

1152 Nicolás, F. E., and R. M. Ruiz-Vázquez. 2013. Functional diversity of RNAi-associated sRNAs
1153 in fungi. *International Journal of Molecular Sciences* 14 (8):15348-15360.

1154 Noë, R., and P. Hammerstein. 1994. Biological markets: supply and demand determine the effect
1155 of partner choice in cooperation, mutualism and mating. *Behavioral Ecology and*
1156 *Sociobiology* 35 (1):1-11.

1157 Nürnberger, T., F. Brunner, B. Kemmerling, and L. Piater. 2004. Innate immunity in plants and
1158 animals: striking similarities and obvious differences. *Immunological Reviews* 198:249-
1159 266.

1160 Öhman, T., L. Teirilä, A.-M. Lahesmaa-Korpinen, W. Cypriak, V. Veckman, S. Saijo, H. Wolff,
1161 S. Hautaniemi, T. A. Nyman, and S. Matikainen. 2014. Dectin-1 pathway activates robust
1162 autophagy-dependent unconventional protein secretion in human macrophages. *Journal*
1163 *of Immunology* 192 (12):5952-5962.

1164 Ong, S. T., J. Z. S. Ho, B. Ho, and J. L. Ding. 2006. Iron-withholding strategy in innate
1165 immunity. *Immunobiology* 211 (4):295-314.

1166 Oozeer, R., K. van Limpt, T. Ludwig, K. Ben Amor, R. Martin, R. D. Wind, G. Boehm, and J.
1167 Knol. 2013. Intestinal microbiology in early life: specific prebiotics can have similar
1168 functionalities as human-milk oligosaccharides. *American Journal of Clinical Nutrition*
1169 98 (2):561S-571S.

1170 Page, R. D. M. 2003. *Tangled Trees: Phylogeny, Cospeciation, and Coevolution*. Chicago: The
1171 University of Chicago Press.

1172 Palaniyandi, S. A., S. H. Yang, L. X. Zhang, and J. W. Suh. 2013. Effects of actinobacteria on
1173 plant disease suppression and growth promotion. *Applied Microbiology and*
1174 *Biotechnology* 97 (22):9621-9636.

1175 Paoletti, M., and S. J. Saupe. 2009. Fungal incompatibility: Evolutionary origin in pathogen
1176 defense? *Bioessays* 31 (11):1201-1210.

1177 Paoletti, M., S. J. Saupe, and C. Clavé. 2007. Genesis of a fungal non-self recognition repertoire.
1178 *Plos One* 2 (3):e283.

1179 Partida-Martinez, L. P., and C. Hertweck. 2005. Pathogenic fungus harbours endosymbiotic
1180 bacteria for toxin production. *Nature* 437 (7060):884-888.

1181 Partida-Martinez, L. P., S. Monajembashi, K. O. Greulich, and C. Hertweck. 2007.
1182 Endosymbiont-dependent host reproduction maintains bacterial-fungal mutualism.
1183 *Current Biology* 17 (9):773-777.

1184 Pawlowska, A. M., E. Zannini, A. Coffey, and E. K. Arendt. 2012. "Green preservatives":
1185 Combating fungi in the food and feed industry by applying antifungal lactic acid bacteria.
1186 In *Advances in Food and Nutrition Research*, edited by J. Henry: Elsevier Inc.

1187 Peleg, A. Y., D. A. Hogan, and E. Mylonakis. 2010. Medically important bacterial–fungal
1188 interactions. *Nature Reviews Microbiology* 8 (5):340-349.

1189 Perotto, S., and P. Bonfante. 1997. Bacterial associations with mycorrhizal fungi: Close and
1190 distant friends in the rhizosphere. *Trends in Microbiology* 5 (12):496-501.

1191 Piispanen, A. E., and D. A. Hogan. 2008. PEPped up: Induction of *Candida albicans* virulence
1192 by bacterial cell wall fragments. *Cell Host & Microbe* 4 (1):1-2.

1193 Pion, M., J. E. Spangenberg, A. Simon, S. Bindschedler, C. Flury, A. Chatelain, R. Bshary, D.
1194 Job, and P. Junier. 2013. Bacterial farming by the fungus *Morchella crassipes*.
1195 *Proceedings of the Royal Society B-Biological Sciences* 280 (1773):20132242.

1196 Pliego, C., C. Ramos, A. de Vicente, and F. M. Cazorla. 2011. Screening for candidate bacterial
 1197 biocontrol agents against soilborne fungal plant pathogens. *Plant and Soil* 340 (1-2):505-
 1198 520.
 1199 Rajamäki, K., T. Nordström, K. Nurmi, K. E. O. Åkerman, P. T. Kovanen, K. Öörni, and K. K.
 1200 Eklund. 2013. Extracellular acidosis is a novel danger signal alerting innate immunity via
 1201 the NLRP3 inflammasome. *Journal of Biological Chemistry* 288 (19):13410-13419.
 1202 Ramírez-Puebla, S. T., L. E. Servín-Garcidueñas, B. Jiménez-Marín, L. M. Bolaños, M.
 1203 Rosenblueth, J. Martínez, M. Antonio Rogel, E. Ormeño-Orrillo, and E. Martínez-
 1204 Romero. 2013. Gut and root microbiota commonalities. *Applied and Environmental*
 1205 *Microbiology* 79 (1):2-9.
 1206 Remis, J. P., D. Wei, A. Gorur, M. Zemla, J. Haraga, S. Allen, H. E. Witkowska, J. W.
 1207 Costerton, J. E. Berleman, and M. Auer. 2014. Bacterial social networks: structure and
 1208 composition of *Myxococcus xanthus* outer membrane vesicle chains. *Environmental*
 1209 *Microbiology* 16 (2):598-610.
 1210 Rodrigues, M. L., E. S. Nakayasu, I. C. Almeida, and L. Nimrichter. 2014. The impact of
 1211 proteomics on the understanding of functions and biogenesis of fungal extracellular
 1212 vesicles. *Journal of Proteomics* 97:177-186.
 1213 Ruiz-Herrera, J., C. León-Ramírez, A. Vera-Núñez, A. Sánchez-Arreguín, R. Ruiz-Medrano, H.
 1214 Salgado-Lugo, L. Sánchez-Segura, and J. J. Peña-Cabriaes. 2015. A novel intracellular
 1215 nitrogen-fixing symbiosis made by *Ustilago maydis* and *Bacillus* spp. *New Phytologist*
 1216 207 (3):769-777.
 1217 Sachs, J. L., U. G. Mueller, T. P. Wilcox, and J. J. Bull. 2004. The evolution of cooperation.
 1218 *Quarterly Review of Biology* 79 (2):135-160.

1219 Saliba, A. E., A. J. Westermann, S. A. Gorski, and J. Vogel. 2014. Single-cell RNA-seq:
 1220 advances and future challenges. *Nucleic Acids Research* 42 (14):8845-8860.
 1221 Salvioli, A., S. Ghignone, M. Novero, L. Navazio, F. Venice, P. Bagnaresi, and P. Bonfante.
 1222 2016. Symbiosis with an endobacterium increases the fitness of a mycorrhizal fungus,
 1223 raising its bioenergetic potential. *ISME Journal* 10 (1):130-144.
 1224 Samuel, M., M. Bleackley, M. Anderson, and S. Mathivanan. 2015. Extracellular vesicles
 1225 including exosomes in cross kingdom regulation: a viewpoint from plant-fungal
 1226 interactions. *Frontiers in Plant Science* 6:766.
 1227 Sato, Y., K. Narisawa, K. Tsuruta, M. Umezu, T. Nishizawa, K. Tanaka, K. Yamaguchi, M.
 1228 Komatsuzaki, and H. Ohta. 2010. Detection of betaproteobacteria inside the mycelium of
 1229 the fungus *Mortierella elongata*. *Microbes and Environments* 25 (4):321-324.
 1230 Scherlach, K., B. Busch, G. Lackner, U. Paszkowski, and C. Hertweck. 2012. Symbiotic
 1231 cooperation in the biosynthesis of a phytotoxin. *Angewandte Chemie* 51 (38):9615-9618.
 1232 Scherlach, K., K. Graupner, and C. Hertweck. 2013. Molecular bacteria-fungi interactions:
 1233 effects on environment, food, and medicine. *Annual Review of Microbiology* 67:375-97.
 1234 Scheublin, T. R., I. R. Sanders, C. Keel, and J. R. van der Meer. 2010. Characterisation of
 1235 microbial communities colonising the hyphal surfaces of arbuscular mycorrhizal fungi.
 1236 *ISME Journal* 4 (6):752-763.
 1237 Schmitt, I., L. P. Partida-Martinez, R. Winkler, K. Voigt, E. Einax, F. Dölz, S. Telle, J.
 1238 Wöstemeyer, and C. Hertweck. 2008. Evolution of host resistance in a toxin-producing
 1239 bacterial-fungal alliance. *ISME Journal* 2 (6):632-641.

1240 Scholtens, P. A. M. J., R. Oozeer, R. Martin, K. B. Amor, and J. Knol. 2012. The early settlers:
 1241 Intestinal microbiology in early life. *Annual Review of Food Science and Technology* 3
 1242 (3):425-447.

1243 Schroeckh, V., K. Scherlach, H.-W. Nützmann, E. Shelest, W. Schmidt-Heck, J. Schuemann, K.
 1244 Martin, C. Hertweck, and A. A. Brakhage. 2009. Intimate bacterial-fungal interaction
 1245 triggers biosynthesis of archetypal polyketides in *Aspergillus nidulans*. *Proceedings of*
 1246 *the National Academy of Sciences of the United States of America* 106 (34):14558-14563.

1247 Schüßler, A., D. Mollenhauer, E. Schnepf, and M. Kluge. 1994. *Geosiphon pyriforme*, an
 1248 endosymbiotic association of fungus and cyanobacteria: the spore structure resembles
 1249 that of arbuscular mycorrhizal (AM) fungi. *Botanica Acta* 107 (1):36-45.

1250 Scully, C., M. el-Kabir, and L. P. Samaranayake. 1994. *Candida* and oral candidosis: A review.
 1251 *Critical Reviews in Oral Biology and Medicine* 5 (2):125-157.

1252 Selin, C., R. Habibian, N. Poritsanos, S. N. P. Athukorala, D. Fernando, and T. R. de Kievit.
 1253 2010. Phenazines are not essential for *Pseudomonas chlororaphis* PA23 biocontrol of
 1254 *Sclerotinia sclerotiorum*, but do play a role in biofilm formation. *FEMS Microbiology*
 1255 *Ecology* 71 (1):73-83.

1256 Seneviratne, G., and H. S. Jayasinghearachchi. 2003. Mycelial colonization by bradyrhizobia and
 1257 azorhizobia. *Journal of Biosciences* 28 (2):243-247.

1258 Seneviratne, G., J. S. Zavahir, W. M. M. S. Bandara, and M. L. M. A. W. Weerasekara. 2008.
 1259 Fungal-bacterial biofilms: their development for novel biotechnological applications.
 1260 *World Journal of Microbiology & Biotechnology* 24 (6):739-743.

1261 Sengupta, S., M. K. Chattopadhyay, and H.-P. Grossart. 2013. The multifaceted roles of
 1262 antibiotics and antibiotic resistance in nature. *Frontiers in Microbiology* 4:47.

1263 Sharma, M., M. Schmid, M. Rothballer, G. Hause, A. Zuccaro, J. Imani, P. Kämpfer, E.
 1264 Domann, P. Schäfer, A. Hartmann, and K. H. Kogel. 2008. Detection and identification
 1265 of bacteria intimately associated with fungi of the order Sebaciales. *Cellular*
 1266 *Microbiology* 10 (11):2235-2246.

1267 Siavoshi, F., and P. Saniee. 2014. Vacuoles of *Candida* yeast as a specialized niche for
 1268 *Helicobacter pylori*. *World Journal of Gastroenterology* 20 (18):5263-73.

1269 Simon, A., S. Bindschedler, D. Job, L. Y. Wick, S. Filippidou, W. M. Kooli, E. P. Verrecchia,
 1270 and P. Junier. 2015. Exploiting the fungal highway: development of a novel tool for the
 1271 *in situ* isolation of bacteria migrating along fungal mycelium. *FEMS Microbiology*
 1272 *Ecology* 91 (11):10.1093/femsec/ v116.

1273 Smith, S. E., and D. J. Read. 2008. *Mycorrhizal Symbiosis*. Third ed. New York: Academic
 1274 Press.

1275 Splivallo, R., A. Deveau, N. Valdez, N. Kirchhoff, P. Frey-Klett, and P. Karlovsky. 2015.
 1276 Bacteria associated with truffle-fruited bodies contribute to truffle aroma. *Environmental*
 1277 *Microbiology* 17 (8):2647-60.

1278 Splivallo, R., S. Ottonello, A. Mello, and P. Karlovsky. 2011. Truffle volatiles: from chemical
 1279 ecology to aroma biosynthesis. *New Phytologist* 189 (3):688-99.

1280 Staněk, M. 1984. Microorganisms in the hyphosphere of fungi. I. Introduction. *Česká Mykologie*
 1281 38 (1):1-10.

1282 Suárez-Moreno, Z. R., J. Caballero-Mellado, B. G. Coutinho, L. Mendonça-Previato, E. K.
 1283 James, and V. Venturi. 2012. Common features of environmental and potentially
 1284 beneficial plant-associated *Burkholderia*. *Microbial Ecology* 63 (2):249-266.

1285 Susi, P., G. Aktuganov, J. Himanen, and T. Korpela. 2011. Biological control of wood decay
 1286 against fungal infection. *Journal of Environmental Management* 92 (7):1681-1689.
 1287 Teichert, I., G. Wolff, U. Kück, and M. Nowrousian. 2012. Combining laser microdissection and
 1288 RNA-seq to chart the transcriptional landscape of fungal development. *BMC Genomics*
 1289 13:511.
 1290 Thompson, J. N. 2005. *The Geographic Mosaic of Coevolution*. Chicago: University of Chicago
 1291 Press.
 1292 Thompson, J. N. 2014. *Interaction and Coevolution*. Chicago: University of Chicago Press.
 1293 Thrane, C., T. H. Nielsen, M. N. Nielsen, J. Sørensen, and S. Olsson. 2000. Viscosinamide-
 1294 producing *Pseudomonas fluorescens* DR54 exerts a biocontrol effect on *Pythium ultimum*
 1295 in sugar beet rhizosphere. *FEMS Microbiology Ecology* 33 (2):139-146.
 1296 Tisserant, E., M. Malbreil, A. Kuo, A. Kohler, A. Symeonidi, R. Balestrini, P. Charron, N.
 1297 Duensing, N. Frei dit Frey, V. Gianinazzi-Pearson, L. B. Gilbert, Y. Handa, J. R. Herr,
 1298 M. Hijri, R. Koul, M. Kawaguchi, F. Krajinski, P. J. Lammers, F. G. Masclaux, C. Murat,
 1299 E. Morin, S. Ndikumana, M. Pagni, D. Petitpierre, N. Requena, P. Rosikiewicz, R. Riley,
 1300 K. Saito, H. San Clemente, H. Shapiro, D. van Tuinen, G. Bécard, P. Bonfante, U.
 1301 Paszkowski, Y. Y. Shachar-Hill, G. A. Tuskan, P. W. Young, I. R. Sanders, B. Henrissat,
 1302 S. A. Rensing, I. V. Grigoriev, N. Corradi, C. Roux, and F. Martin. 2013. Genome of an
 1303 arbuscular mycorrhizal fungus provides insight into the oldest plant symbiosis.
 1304 *Proceedings of the National Academy of Sciences of the United States of America* 110
 1305 (50):20117-20122.
 1306 Toomer, K. H., X. Chen, M. Naito, S. J. Mondo, H. C. den Bakker, N. W. VanKuren, Y.
 1307 Lekberg, J. B. Morton, and T. E. Pawlowska. 2015. Molecular evolution patterns reveal

1308 life history features of mycoplasma-related endobacteria associated with arbuscular
 1309 mycorrhizal fungi. *Molecular Ecology* 24 (13):3485-3500.
 1310 Tornberg, K., E. Bååth, and S. Olsson. 2003. Fungal growth and effects of different wood
 1311 decomposing fungi on the indigenous bacterial community of polluted and unpolluted
 1312 soils. *Biology and Fertility of Soils* 37 (3):190-197.
 1313 Torres-Cortés, G., S. Ghignone, P. Bonfante, and A. Schüßler. 2015. Mosaic genome of
 1314 endobacteria in arbuscular mycorrhizal fungi: Transkingdom gene transfer in an ancient
 1315 mycoplasma-fungus association. *Proceedings of the National Academy of Sciences of the*
 1316 *United States of America* 112 (25):7785-7790.
 1317 Trinchieri, G. 2014. Cancer as a disease of the metaorganism. *Immunology* 143 (Suppl 2):13.
 1318 Trivers, R. L. 1971. The evolution of reciprocal altruism. *Quarterly Review of Biology* 46:35–57.
 1319 Uzum, Z., A. Silipo, G. Lackner, A. De Felice, A. Molinaro, and C. Hertweck. 2015. Structure,
 1320 genetics and function of an exopolysaccharide produced by a bacterium living within
 1321 fungal hyphae. *ChemBioChem* 16 (3):387-92.
 1322 Val, F., S. Desender, K. Bernard, P. Potin, G. Hamelin, and D. Andrivon. 2008. A culture filtrate
 1323 of *Phytophthora infestans* primes defense reaction in potato cell suspensions.
 1324 *Phytopathology* 98 (6):653-658.
 1325 Vannini, C., A. Carpentieri, A. Salvioli, M. Novero, M. Marsoni, L. Testa, M. C. De Pinto, A.
 1326 Amoresano, F. Ortolani, M. Bracale, and P. Bonfante. 2016 An interdomain network:
 1327 The endobacterium of a mycorrhizal fungus promotes antioxidative responses in both
 1328 fungal and plant hosts. *New Phytologist* doi: 10.1111/nph.13895.
 1329 Vasilyeva, N. V., I. M. Tsfasman, I. V. Kudryakova, N. E. Suzina, N. A. Shishkova, I. S. Kulaev,
 1330 and O. A. Stepnaya. 2013. The role of membrane vesicles in secretion of *Lysobacter* sp

1331 bacteriolytic enzymes. *Journal of Molecular Microbiology and Biotechnology* 23 (1-
1332 2):142-151.

1333 Verma, N., and B. C. Behera. 2015. *In vitro* culture of lichen partners: Need and implications. In
1334 *Recent Advances in Lichenology: Modern Methods and Approaches in Biomonitoring*
1335 *and Bioprospection*, edited by D. K. Upreti, P. K. Divakar, V. Shukla and R. Bajpai. New
1336 Dehli: Springer India.

1337 Wang, X., G. H. Li, C. G. Zou, X. L. Ji, T. Liu, P. J. Zhao, L. M. Liang, J. P. Xu, Z. Q. An, X.
1338 Zheng, Y. K. Qin, M. Q. Tian, Y. Y. Xu, Y. C. Ma, Z. F. Yu, X. W. Huang, S. Q. Liu, X.
1339 M. Niu, J. K. Yang, Y. Huang, and K. Q. Zhang. 2014. Bacteria can mobilize nematode-
1340 trapping fungi to kill nematodes. *Nature Communications* 5:5776.

1341 Warmink, J. A., R. Nazir, B. Corten, and J. D. van Elsas. 2011. Hitchhikers on the fungal
1342 highway: The helper effect for bacterial migration via fungal hyphae. *Soil Biology &*
1343 *Biochemistry* 43 (4):760-765.

1344 Warmink, J. A., R. Nazir, and J. D. van Elsas. 2009. Universal and species-specific bacterial
1345 'fungiphiles' in the mycospheres of different basidiomycetous fungi. *Environmental*
1346 *Microbiology* 11 (2):300-12.

1347 Watrous, J. D., T. Alexandrov, and P. C. Dorrestein. 2011. The evolving field of imaging mass
1348 spectrometry and its impact on future biological research. *Journal of Mass Spectrometry*
1349 46 (2):209-222.

1350 Weyl, E. G., M. E. Frederickson, D. W. Yu, and N. E. Pierce. 2010. Economic contract theory
1351 tests models of mutualism. *Proceedings of the National Academy of Sciences of the*
1352 *United States of America* 107 (36):15712-15716.

1353 Wick, L. Y., R. Remer, B. Wuerz, J. Reichenbach, S. Braun, F. Schaerfer, and H. Harms. 2007.
1354 Effect of fungal hyphae on the access of bacteria to phenanthrene in soil. *Environmental*
1355 *Science & Technology* 41 (2):500-505.

1356 Winsor, G. L., B. Khaira, T. Van Rossum, R. Lo, M. D. Whiteside, and F. S. Brinkman. 2008.
1357 The *Burkholderia* Genome Database: facilitating flexible queries and comparative
1358 analyses. *Bioinformatics* 24 (23):2803-4.

1359 Xu, X. L., R. T. H. Lee, H. M. Fang, Y. M. Wang, R. Li, H. Zou, Y. Zhu, and Y. Wang. 2008.
1360 Bacterial peptidoglycan triggers *Candida albicans* hyphal growth by directly activating
1361 the adenylyl cyclase Cyr1p. *Cell Host & Microbe* 4 (1):28-39.

1362 Yamamura, N. 1993. Vertical transmission and evolution of mutualism from parasitism.
1363 *Theoretical Population Biology* 44 (1):95-109.

1364 Yoshida, S., A. Ohba, Y.-M. Liang, M. Koitabashi, and S. Tsushima. 2012. Specificity of
1365 *Pseudomonas* isolates on healthy and *Fusarium* head blight-infected spikelets of wheat
1366 heads. *Microbial Ecology* 64 (1):214-225.

1367 Young, D. B., I. Comas, and L. P. de Carvalho. 2015. Phylogenetic analysis of vitamin B12-
1368 related metabolism in *Mycobacterium tuberculosis*. *Frontiers in Molecular Biosciences*
1369 2:6.

1370 Yu, F., K. Zaleta-Rivera, X. Zhu, J. Huffman, J. C. Millet, S. D. Harris, G. Yuen, X.-C. Li, and
1371 L. Du. 2007. Structure and biosynthesis of heat-stable antifungal factor (HSAF), a broad-
1372 spectrum antimycotic with a novel mode of action. *Antimicrobial Agents and*
1373 *Chemotherapy* 51 (1):64-72.

1374 Zamioudis, C., and C. M. J. Pieterse. 2012. Modulation of host immunity by beneficial microbes.
1375 *Molecular Plant-Microbe Interactions* 25 (2):139-150.

1376 Zhang, M., and R. Schekman. 2013. Unconventional secretion, unconventional solutions.
1377 *Science* 340 (6132):559-561.
1378

1379
1380 **Table 1.** Mechanisms shared by diverse eukaryotic hosts to select beneficial organisms
1381 colonizing host surfaces involved in nutrient uptake.

General for eukaryotic hosts (means)	Host specific (means)
pH reduction by the host (secretion of hydrogen ion)	Secreted antibacterial compounds (production and secretion of secondary metabolites and/or antimicrobial peptides, AMPs)
Host reduction of iron availability (activation of host iron uptake machinery)	Provisioning of beneficial bacteria with specific nutrients not common in other environments. (synthesis and secretion of specific carbon sources)

1382
1383 **Figure Captions**

1384 **Figure 1. Metaorganisms comprise fungal hosts and their various bacterial symbionts.**

1385

1386 **Figure 2. Evolutionary theory predictions on the role of vertical transmission in the**
1387 **evolution of mutualisms from antagonisms.** Hosts are depicted as red ovals; host-positive
1388 symbionts are shown as green dots, host-negative symbionts as purple dots. Relative host fitness
1389 is reflected by the size of ovals.

1390

1391 **Figure 3. Hypothetical evolutionary trajectories in heritable mutualisms.** Hosts are
1392 depicted as red ovals; endosymbionts are shown as green dots. Relative host fitness is reflected
1393 by the size of ovals. (A) Evolutionary trajectory leading to obligate reciprocal partner
1394 dependence. (B) Shifting environmental conditions are expected to arrest an association at the
1395 facultative dependence stage. If conditions remain unfavorable for prolonged periods of time,
1396 host populations would be expected to completely lose endosymbionts. Modified from Mondo et
1397 al. (2012).

1398

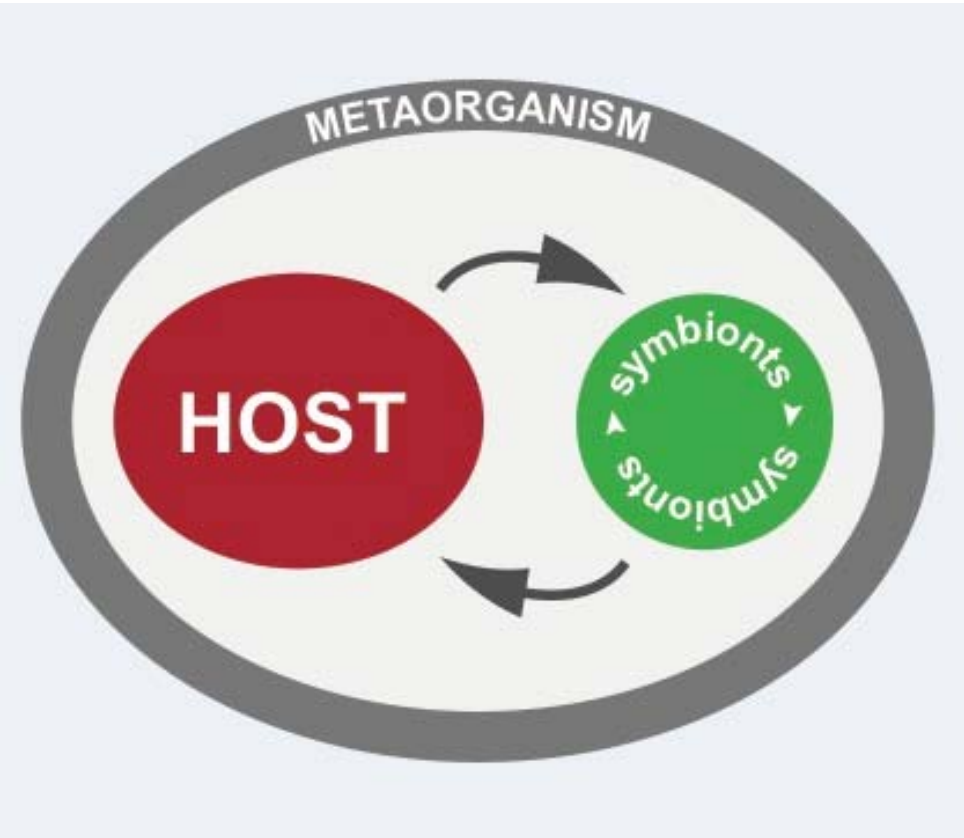
1399 **Figure 4. Model of plant-fungus-endobacterium interaction** (Courtesy of M. Novero).
1400 Genome-sequencing results for *Candidatus Glomeribacter gigasporarum* indicate that the
1401 bacterium fully depends on the fungal metabolism, including carbon (C), phosphorus (P), and
1402 nitrogen (N) metabolism. In contrast, the fungus depends on its green plant host for C uptake
1403 only.

1404

1405 **Figure 5. Evolutionary theory predictions linking the type of symbiosis with the intra-host**
1406 **relatedness of symbionts and symbiont transmission.** Hosts are shown as red ovals. Relative
1407 host fitness is reflected by the size of ovals. Endosymbionts are represented by green and purple
1408 dots with different shades depicting different genotypes. Modified from Toomer et al. (2015).

1409

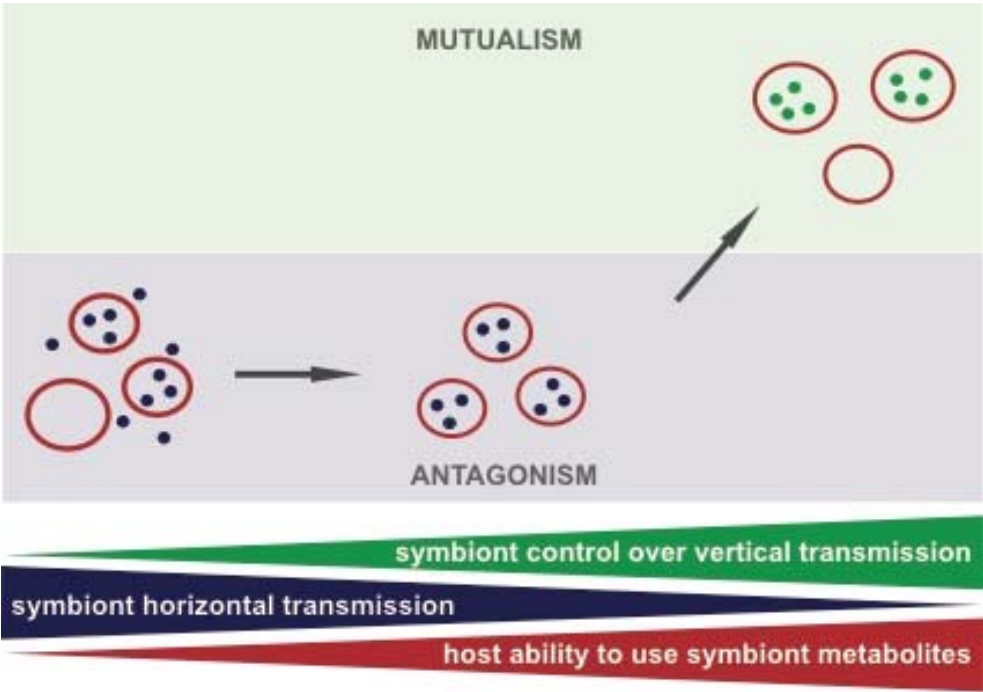
1410 Figure 1



1411

1412

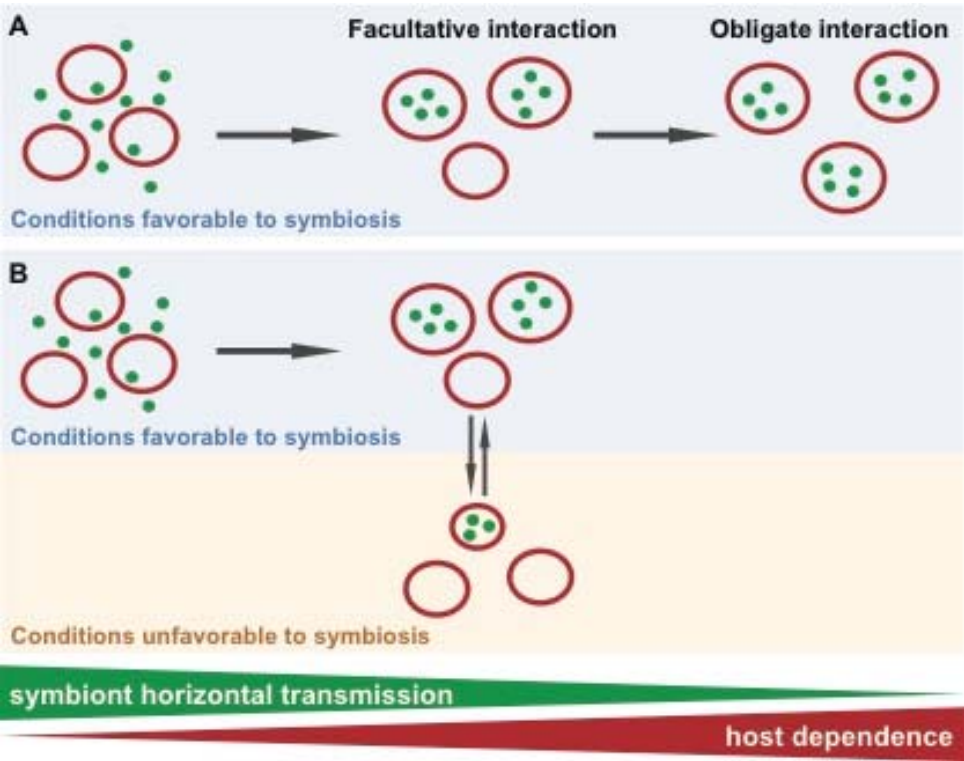
1413 Figure 2



1414

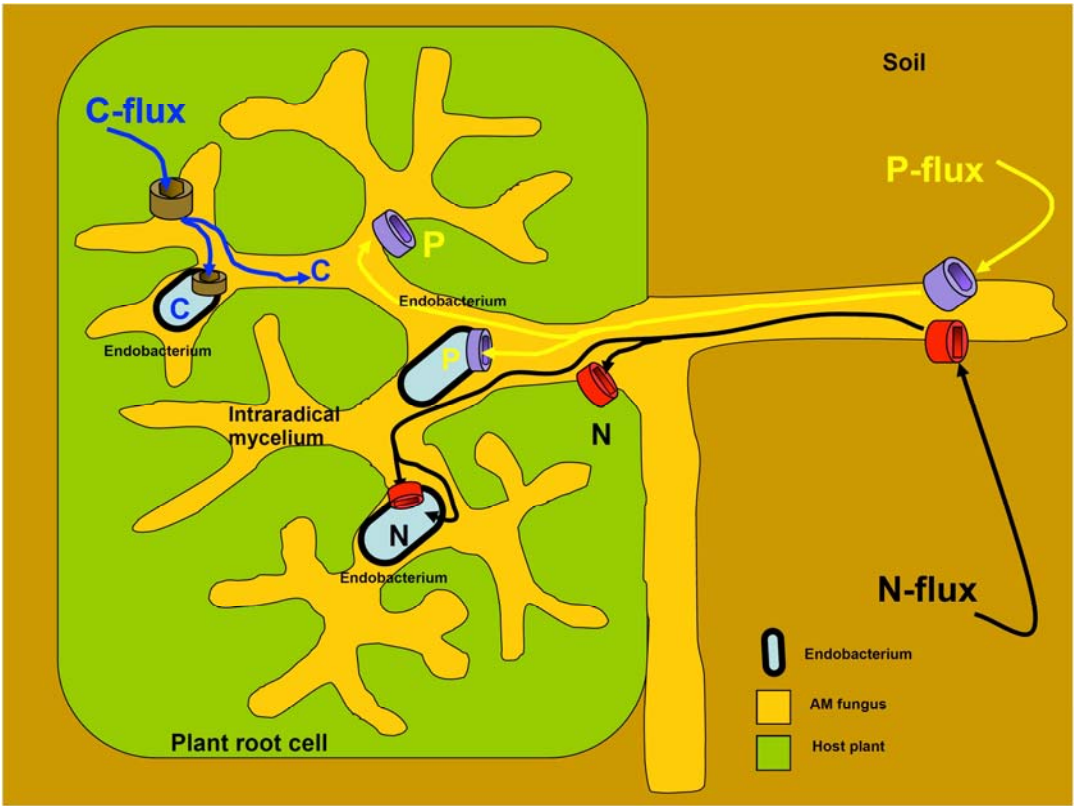
1415

1416 Figure 3



1417

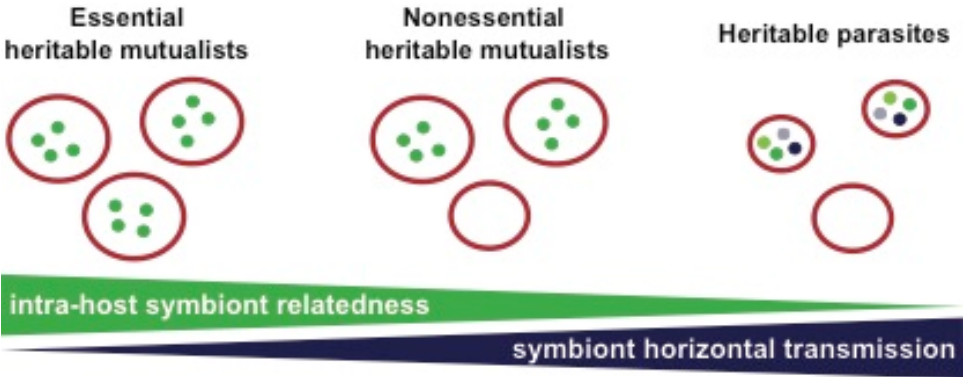
1418
1419 Figure 4



1420

1421

1422 Figure 5



1423